CENTER FOR INFECTIOUS MEDICINE (CIM),
DEPARTMENT OF MEDICINE, KAROLINSKA INSTITUTET,
STOCKHOLM, SWEDEN

ON THE ROLE OF DENDRITIC CELLS IN HIV-1 INFECTION

ANNA SMED SÖRENSEN

stockholm 2004

Anna Smed Sörensen
On the role of dendritic cells in HIV-1 infection
Karolinska Institutet, Stockholm, 2004

This work was funded by grants from the Swedish Research Council; the Swedish Foundation for Strategic Research; Swedish Cancer Society; the Swedish Society of Medicine; Swedish International Development Cooperation Agency/Dept. for Research Cooperation; the Swedish Physicians Against AIDS research foundation and the Tore Nilsson, Magnus Bergvall, Axel and Margaret Johnson and Adolf Lindgren foundations and NIH intramural research funds.

Graphic production Sara Smed Sörensen
Printed by Repro Print AB, Stockholm, 2004

ISBN 91-7140-052-4

To my family

Abstract

Dendritic cells (DCs) are antigen-presenting cells with the capacity to initiate primary T cell responses against pathogens such as HIV-1. In addition, DCs express the receptors required for HIV-1 infection and as DCs are prevalent in the mucosa, they encounter the virus early after sexual transmission. DCs also express additional receptors able to bind and capture intact HIV-1 without becoming infected. Normally, DCs capture antigen in the periphery and migrate to draining lymph nodes to present the antigen to T cells to start immune responses. HIV-1 may utilize this process as, DCs can collect and carry HIV-1 and facilitate spread to CD4+ T cells. To optimize the T cell activation, DCs mature by upregulation of MHC and co-stimulatory molecules and induction of cytokine production. However, another consequence of efficient interaction between DCs and T cells is that it provides an optimal milieu for HIV-1 transmission and replication.

We studied the effects of HIV-1 infection on DC function. We found that monocyte-derived DCs (MDDCs) were productively infected by HIV-1 after in vitro exposure (as measured by intracellular production of HIV-1 p24). HIV-1 infected MDDCs upregulated co-stimulatory molecules in response to CD40ligand stimulation, comparable to uninfected MDDCs. However, intracellular cytokine staining revealed that while the HIV-1 infected DCs were able to produce TNFα, they failed to express IL-12 p70. This may impact the ability of DCs to induce optimal HIV-1 specific immune responses, as IL-12 is vital for the induction of cellular immune responses. Next, we expanded the studies by examining isolated primary myeloid DCs (MDCs) and plasmacytoid DCs (PDCs). MDCs and PDCs became productively infected by different HIV-1 isolates. The DC subsets displayed differential susceptibility to HIV-1. HIV-1 exposure induced some maturation in the DCs. However, TLR ligation induced full maturation and TNFα production in both uninfected and infected DCs. Productively infected MDCs and PDCs efficiently transferred HIV-1 to autologous CD4+ T cells, and antigen-reactivated T cells were more frequently infected than non-responding T cells. This suggests that induction of DC-dependent antigen-specific T cell responses, crucial to the immune defence, also can lead to preferential HIV-1 infection of responding T cell clones in infected individuals.

DCs can present antigens derived from apoptotic cells. We investigated whether apoptotic HIV-1 infected cells were capable of eliciting HIV-1 specific immune responses in vivo. Immunization of mice with apoptotic HIV-1/MuLV infected cells resulted in induction of HIV-1 specific T cell and antibody responses. Moreover, immunized mice handled challenge with live HIV-1/MuLV infected cells more effectively than nonimmunized mice. These data show that immunization of mice with apoptotic HIV-1 infected cells can induce high levels of HIV-1 specific systemic immunity and prime for mucosal immunity that could provide means for the mice to cope with challenge.

Collectively, our findings demonstrate that DCs are under certain conditions impaired by HIV-1 infection but that they efficiently transfer HIV-1 to CD4+ T cells. Taken together, an increased understanding of DC immunobiology may help us develop more effective HIV-1 therapy and/or an HIV-1 vaccine.

Keywords: dendritic cell, plasmacytoid, myeloid, HIV-1, cytokines, co-stimulation, transmission, antigen presentation, apoptotic cells, MuLV.
ISBN: 91-7140-052-4

Original papers

This thesis is based on the following original papers, which are referred to in the text by their Roman numerals:

I.

Anna Smed Sörensen, Karin Loré, Lilian Walther-Jallow, Jan Andersson and Anna-Lena Spetz: HIV-1 infected dendritic cells upregulate cell surface markers but fail to produce IL-12 p70 in response to CD40ligand stimulation.
Blood 2004; 104: 2810.

II.

Anna Smed Sörensen, Karin Loré, Jayanand Vasudevan, Mark K. Louder, John R. Mascola, Jan Andersson, Anna-Lena Spetz and Richard A. Koup: Differential susceptibility to HIV-1 infection of myeloid and plasmacytoid dendritic cells. *Submitted*.

III.

Karin Loré, **Anna Smed Sörensen**, Jayanand Vasudevan, John R. Mascola and Richard A. Koup: Productively infected myeloid and plasmacytoid dendritic cells transfer HIV-1 preferentially to antigen-specific CD4+ T cells.
Manuscript.

IV.

Anna-Lena Spetz, **Anna Smed Sörensen**, Lilian Walther-Jallow, Bruce K. Patterson, Britta Wahren, Jan Andersson, Lars Holmgren and Jorma Hinkula: Induction of HIV-1 specific immunity after vaccination with apoptotic HIV-1/Murine Leukemia Virus infected syngeneic cells.
Journal of Immunology 2002; 169: 5771.

Contents

Abbreviations

Acquired Immunodeficiency Syndrome	AIDS
Antiretroviral Treatment	ART
Azidothymidine	AZT
CC Chemokine Receptor	CCR
Cluster of Differentiation	CD
CD40ligand	CD40L
Carboxy-Fluorescein Diacetate Succinimidyl Ester	CFSE
C-type Lectin Receptor	CLR
Cytomegalovirus	CMV
Cytotoxic T Lymphocyte	CTL
CXC Chemokine Receptor	CXCR
Dendritic Cell	DC
DC-specific ICAM-3 Grabbing Nonintegrin	DC-SIGN
Dermal Dendritic Cell	DDC
Deoxyribonucleic Acid	DNA
Deoxynucleotide Triphosphate	dNTP
Epstein Barr Virus	EBV
Granulocyte/Macrophage-Colony Stimulating Factor	GM-CSF
Hepatits C Virus	HCV
Human Immunodeficiency Virus	HIV
Human Leukocyte Antigen	HLA
Human T cell Leukemia Virus	HTLV
Intercellular Adhesion Molecule	ICAM
Interferon	IFN
Immunoglobulin	Ig
Interleukin	IL
Langerhans Cell	LC
Lipopolysaccaride	LPS
Myeloid Dendritic Cell	MDC
Monocyte-Derived Dendritic Cell	MDDC
Major Histocompatibility Complex	MHC
Mixed Lymphocyte Reaction	MLR
Mannose Receptor	MMR
Murine Leukemia Virus	MuLV
Pathogen-Associated Molecular Patterns	PAMP
Peripheral Blood Mononuclear Cell	PBMC
Plasmacytoid Dendritic Cell	PDC
Ribonucleic Acid	RNA
Reverse Transcriptase	RT
Staphylococcal Enterotoxin B	SEB
Simian Immunodeficiency Virus	SIV
Structured Treatment Interruption	STI
Transforming Growth Factor	TGF
Toll-Like Receptor	TLR
Tumor Necrosis Factor	TNF

Aims of this thesis

The general aim of this thesis was to study the effects of HIV-1 infection on DC function, as these effects are poorly understood but most likely central to our understanding of HIV-1 disease pathogenesis. Furthermore, we investigated the potential use of apoptotic HIV-1 infected cells as a vaccine. The specific objectives were:

To study the ability of HIV-1_{BaL} infected MDDCs to upregulate co-stimulatory molecules and produce cytokines in response to CD40L stimulation (**paper I**).

To assess the susceptibility of primary MDCs and PDCs to HIV-1_{BaL} and HIV-1_{IIIB} infection, and the capacity of these cells to upregulate co-stimulatory molecules and produce cytokines in response to TLR stimulation after HIV-1 infection (**paper II**).

To study the transfer of HIV-1_{BaL} and HIV-1_{IIIB} from infected primary MDCs and PDCs to autologous CD4+ T cells during antigen presentation, with focus on transmission efficacy and the nature of the T cells infected by DCs (**paper III**).

To determine whether apoptotic HIV-1 infected cells are capable of eliciting HIV-1 specific immune responses in vivo (**paper IV**).

Dendritic cells

Dendritic cells were first described in the human skin by the German medical student Paul Langerhans, who assumed that they were nerve cells based on their morphology, and published his observations on Langerhans cells (LCs) in 1868 (1). Later, the identification of cytoplasmic organelles termed Birbeck granules, which are unique for LCs, allowed further characterization of these cells (2). The function of Birbeck granules remains elusive but they are suggested to be involved in the endocytic pathway of LCs (3-5). Cells containing Birbeck granules were identified in the lymph, lymph nodes and thymus (6-8). It was further shown that these cells expressed major histocompatibility complex (MHC) class II on their cell surface (9-11), and were capable of antigen presentation (12) and lymphocyte activation (13-15). However, the discovery of a small population of “large stellate” cells named dendritic cells (DCs) in peripheral lymphoid organs of mice by Steinman and Cohn in 1973 (16-19), reinitiated modern DC research. It was established that LCs and DCs in the skin as well as in the thymus, are not nerve cells but originate from hematopoetic progenitors of the bone marrow (20-22).

Dendritic cell development

DCs are quite rare cells (1-3% of all skin cells and < 1% of peripheral blood mononuclear cells (PBMCs)), and laborious to isolate. Thus, the establishment of protocols to generate DCs in vitro from progenitors has had a considerable effect on the ability to study these cells. It was first shown that mouse blood and bone marrow contain progenitors that can develop into DCs when cultured in the presence of GM-CSF (23, 24). Also in the human setting, it is possible to generate large numbers of DC-like cells by culturing progenitors from bone marrow, cord blood and peripheral blood with specific cytokines (25-29). A lot of our current knowledge on the development and functions of human DCs comes from studies on monocyte-derived DCs (MDDCs), where peripheral blood monocytes are cultured in the presence of GM-CSF and IL-4 to give rise to DCs (26, 28) that resemble dermal DCs (30). The addition of TGFβ to progenitor cultures promotes development of cells that closely resemble LCs, with expression of Birbeck granules (31, 32).

Despite the central role of GM-CSF in in vitro culture systems, mice deficient in GM-CSF or its receptor still produce DCs (33). Increasing the levels of GM-CSF in mice results in only a small increase in the number of DCs (33), while another cytokine Flt3 ligand (a stimulus for growth and differentiation of early hematopoietic progenitors) substantially increases the number of circulating DCs, both in humans and mice (34-36). This indicates that some DC subsets found in vivo are less dependent on GM-CSF compared to MDDCs and also that other stimuli can induce DC differentiation. Data to support the use of MDDCs as an in vitro model was reported by Randolph and co-workers, who showed that human monocytes cultured with endothelium differentiated into DCs within 2 days without the addition of cytokines. Monocytes were shown to cross a layer of endothelial cells and enter a collagen matrix, mimicking the entry of monocytes into tissue from the blood stream. A proportion of these monocyte-derived cells migrated back across the endothelium, in parallel with DCs migrating from the tissue to the lymph. The cells that remained in the tissue-like matrix became macrophages, while those that migrated were identified as DCs. This phenomenon was enhanced if the cells actively phagocytosed material in the collagen matrix (37). These data provide the first direct evidence that human blood monocytes can be induced to become mature DCs in the absence of exogenous cytokines.

Dendritic cell subsets

DCs constitute a heterogeneous population of specialized bone-marrow derived leukocytes. Different subsets of DCs are distributed throughout the body and can be found in almost all tissues (38) (Figure 1). The majority of DC subsets described in various tissues is of myeloid origin and express myeloid markers like CD11c, CD11b and CD33 but lack expression of other lineage markers like CD3, CD19, CD20, CD14 and CD56 (39). In addition, a second lymphoid-related DC differentiation pathway was recently described, that give rise to plasmacytoid DCs (PDCs) (40). PDCs morphologically resemble antibody-secreting plasma cells, and secrete considerable levels of type I interferons in response to exposure of viruses or microbial components (41, 42). PDCs possess characteristics common to all DCs; high expression of MHC and co-stimulatory molecules upon activation and the capacity to induce T cell proliferation (43). However, PDCs do not express myeloid markers and are in humans often defined by their expression of CD123 (the α chain

of the IL-3 receptor) and lack of CD11c. PDCs are sparsely distributed and only found in blood and lymphoid tissues, like thymus, tonsils and spleen (40, 44-47). Another DC subset also found in peripheral blood is myeloid DCs (MDCs). MDCs are widely distributed and are found throughout the body. LCs reside in the epidermis and dermal DCs (DDCs) are found in the dermis of the skin. Similar DC subsets of myeloid origin exist in the mucosal epithelia, and are then termed mucosal DCs and submucosal DCs, respectively. As described earlier, LCs are distinguished by Birbeck granules, and they also express langerin, CD1a and CD1c in addition to myeloid markers like CD11b and CD33 (48). DDCs share most of the phenotype of LCs but lack expression of langerin and Birbeck granules. In addition, DDCs express CD1b and in some cases the monocyte/macrophage marker CD14 (49-51).

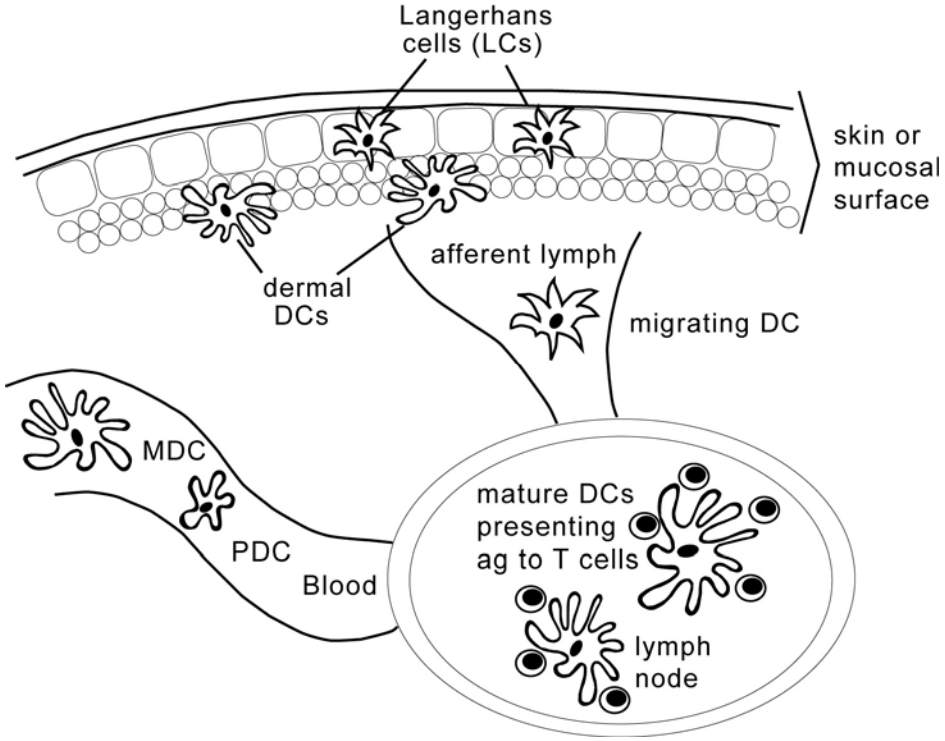

Figure 1. *DC subset distribution. Immature Langerhans cells (LCs) reside in the epidermis of the skin and immature dermal DCs are found in the dermis. Similar DC subsets exist in the mucosa, termed mucosal DCs and submucosal DCs, respectively. Both immature myeloid DCs (MDCs) and plasmacytoid DCs (PDCs) exist in peripheral blood. Upon antigen encounter all DCs subsets mature and migrate to draining lymph nodes, where mature DCs present the antigen to T cells.*

Several subsets of DCs have been described, but whether each subset has a particular function in terms of mounting immune responses against various pathogens is only beginning to be unravelled. The fact that the immune system is constituted by multiple DC subsets with distinct anatomical locations and expression of toll-like receptors (TLRs) used to recognize pathogens (described below), could suggest that the various DC populations specialize in handling certain types of pathogens.

Recognition and presentation of antigens

Immature DCs migrate via the blood into peripheral tissues where they encounter foreign antigen. To assist recognition, DCs express receptors that bind conserved structures commonly found on pathogens. Binding of foreign structures to these receptors facilitate maturation of DCs that present the antigens, which can help induce proper immune responses in order to combat the particular pathogen. DCs express TLRs that recognize conserved structures, known as pathogen-associated molecular patterns (PAMPs), that are unique to the microbial world and found on entire classes of pathogens (52, 53) (Figure 2).

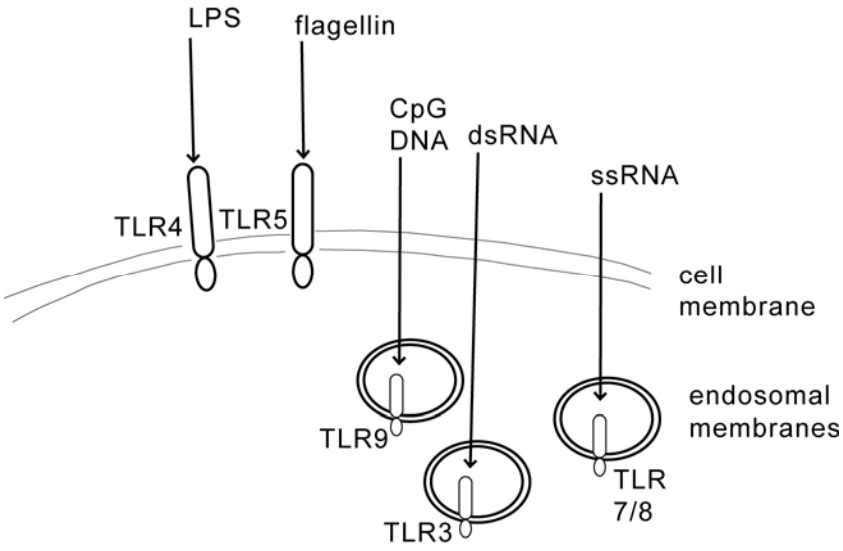

Figure 2. *Various toll-like receptors and their ligands. TLRs 4 and 5 expressed on the cell surface recognize bacterial LPS and flagellin, respectively. In contrast, TLRs 3, 7/8 and 9 recognize nucleic acids (CpG DNA, dsRNA, ssRNA) mainly originating from viral pathogens. These latter TLRs are therefore localized intracellularly and detect nucleic acids in compartments normally not accessible to the nucleic acids derived from the host.*

When TLR ligands bind their receptors, an intracellular signaling cascade induces DC maturation as defined by upregulation of MHC class I and II, increased expression of co-stimulatory molecules and secretion of cytokines.

Human DC subsets express distinct patterns of TLRs and may subsequently be suited to confront different pathogens and dictate immune responses accordingly (41, 42) (Figure 3). TLR3 recognizes double-stranded RNA and is expressed on MDCs (54), while TLR9, expressed by PDCs, interacts with unmethylated bacterial DNA with immunostimulatory CG motifs, known as CpG oligodeoxynucleotides (55, 56). MDCs also express low levels of TLR4 and TLR5 that bind LPS and flagellin, respectively (41, 42). Furthermore, MDCs and PDCs express TLR7 and TLR8 that both recognize single-stranded RNA and imidazoquinoline compounds, like imiquimod and R-848 (57-61).

In addition, DCs express C-type lectin receptors (CLRs). CLRs bind sugars in a calcium-dependent manner using highly conserved carbohydrate recognition domains (62).

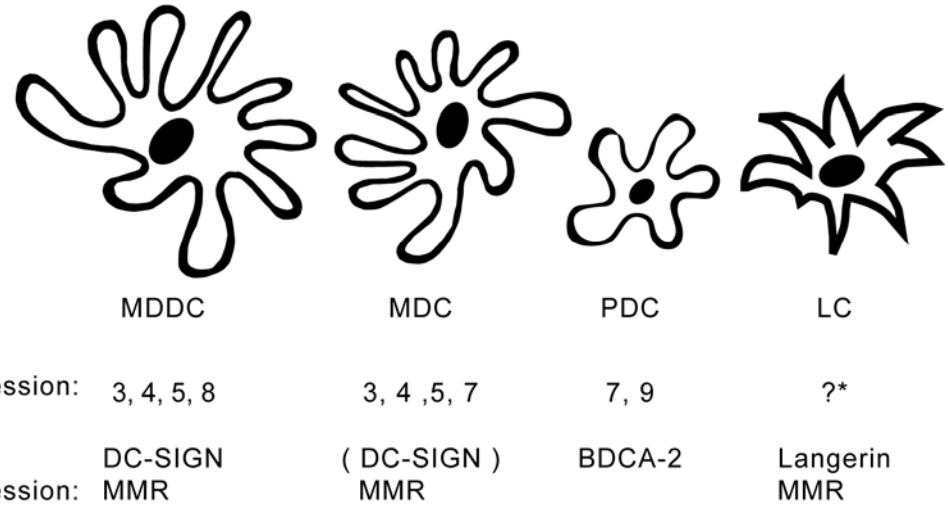

Figure 3. *Expression of some TLRs and CLRs on different DC subsets. *The expression of TLRs on LCs is poorly characterized. However, LCs respond to stimulation with bacterial peptidoglycan (TLR2), LPS (TLR4) and flagellin (TLR5) (63).*

Binding of antigens to CLRs can facilitate internalization of the antigens for degradation and presentation by DCs (64, 65). However, CLRs do not only function as antigen receptors, but also regulate migration of DCs and their interaction with T

cells (66, 67). Different subsets of DCs express different CLRs, like the macrophage mannose receptor (MMR/CD206) on MDDCs and DDCs, Langerin (CD207) on LCs, DC-SIGN (CD209) on MDDCs, blood MDCs and some DDCs (49, 64, 68, 69) (Figure 3). While TLRs bind structures mainly found on non-self antigens, CLRs bind sugar domains found frequently on self antigens, but also on pathogens. Although most CLRs bind mannosylated antigens, their specific self ligands are not completely defined. MMR is known to bind mannose and fucose and DC-SIGN binds mannan, ICAM-2 and ICAM-3, while any self ligand for Langerin has not been identified. A number of CLRs are known to bind gp120 on the human immunodeficiency virus type 1 (HIV-1), like DC-SIGN, MMR and Langerin (70, 71). DC-SIGN has also been described to bind several other pathogens, including simian immunodeficiency virus (SIV) (72), Ebola virus (73, 74), cytomegalovirus (CMV) (75), hepatits C virus (HCV) (76-78), *Mycobacterium tuberculosis* (79, 80) and *Candida albicans* (81). New data indicate that there is a cross-talk between TLRs and CLRs that can affect the type of immune response induced by the DCs (82-85).

Upon recognition, DCs are able to take up antigens using several mechanisms, including receptor-mediated endocytosis, phagocytosis and macropinocytosis. They subsequently process the antigens to peptides that are loaded onto MHC class I and II molecules (86). The intracellular pathways for antigen processing and peptide loading on MHC class I and II molecules have been well characterized and are reviewed in detail elsewhere (86-89). In general, endogenous antigens are restricted to presentation on MHC class I and recognition by CD8+ T cells, while exogenous antigens are presented on MHC class II for the recognition by CD4+ T cells. However, in 1976 Michael Bevan demonstrated a phenomenon he termed cross-priming, priming of class I restricted CD8+ T cells as a consequence of presentation of exogenous antigens on MHC class I (90). The ability of DCs and other antigen-presenting cells to present internalized antigens on MHC class I molecules is referred to as cross-presentation (91).

Dendritic cell migration and maturation

As described above, DCs are distributed throughout the body. DCs are often defined as immature and mature, based on their function and expression of cell surface markers and cytokine production. This definition also reflects their location in vivo, as

the functions of immature and mature DCs are linked to their physical compartmentalization in the body (92) (Figure 4). Upon encounter with antigen and possibly signaling via TLRs, DCs migrate to draining lymph nodes. The migratory capacity of DCs was first demonstrated by staining the skin of mice with fluorescein isothiocyanate (FITC) and proving that FITC was concentrated to the DCs, which migrated to and remained in the draining lymph nodes. This demonstrated the relationship between skin DCs and lymph node DCs (93, 94).

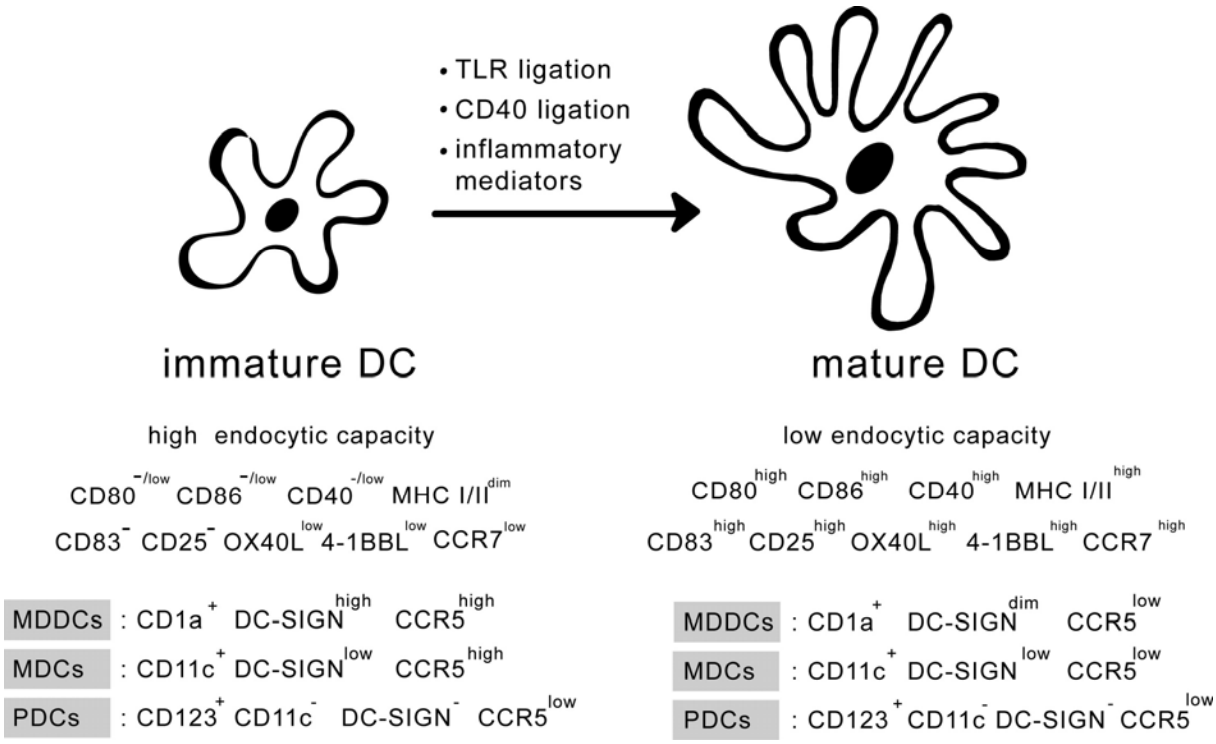

Figure 4. *Some phenotypic and functional properties of immature and mature DCs.*

The expression of chemokine receptors is important for cells to migrate in response to chemokines secreted at sites of inflammation or in lymphoid compartments. The repertoire of chemokine receptors on DCs change throughout maturation, which affects their responsiveness to specific chemokines and facilitate migration (95, 96). Immature DCs express the chemokine receptors CCR1, CCR2, CCR5 and CXCR1 and respond to their respective ligands; MIP-1α, MCP-1, MIP-1β and IL-8, respectively, which are chemokines produced at inflammatory sites. Upon maturation, DCs downregulate CCR1, CCR5 and CXCR1 and instead express high levels of the homing receptor CCR7. One of the ligands of CCR7 is CCL19, which is

produced in lymphoid organs and is believed to facilitate DC migration to T cell areas of the lymphoid tissues (95, 96).

Not only the expression of chemokine receptors changes in response to antigen, but DCs undergo a series of maturational changes and become efficient antigen-presenting cells. Maturation of DCs and subsequent reduction of endocytic capacity, often call for additional stimuli like inflammatory cytokines or microbial products in addition to antigen uptake. A recent report shows, that the antigen capturing capacity of DCs is transiently increased immediately after TLR ligand stimulation and results in enhanced presentation of antigen on MHC class I and II (97).

Multiple receptors are described to be able to trigger DC maturation upon ligation, like TLRs, cytokine receptors, members of the TNF receptor family and Fc receptors (86). As described previously, DCs express TLRs that recognize various pathogenic compounds, like LPS (TLR4), CpG (TLR9) and double-stranded RNA (TLR7). These substances, or other TLR binding compounds like R-848 (TLR7/8), are frequently used in vitro to induce and study DC maturation (41, 43, 55, 59). DCs also sense infections indirectly by responding to inflammatory mediators like TNF, IL-1β and PGE2, which are often elevated in tissues in response to pathogens (98, 99). Another potent inducer of DC maturation is signaling of CD40 on DCs by its ligand CD40ligand (CD40L or CD154). CD40L is expressed mainly expressed on activated T cells (100). Also binding of other molecules present on T cells, like OX40 and FasL to their corresponding ligands on DCs, OX40L and Fas can induce DC maturation. In addition, DCs may also be activated trough immunoglobulin Fc receptors by engagement of immune complexes or specific antibodies (101-103).

Upon signaling by any of the receptors mentioned, DCs upregulate the expression of a panel of molecules on their cell surface. MHC class II is upregulated, to facilitate good presentation of processed antigens (104). In addition, the expression of co-stimulatory molecules like CD80, CD86, CD83, CD40, OX40L and 4-1BBL increase to support adequate cross talk with T cells (28, 105-108) (Figure 5). Mature DCs also start to produce and secrete numerous pro-inflammatory cytokines, which are central in orchestrating the subsequent cellular immune response.

Dendritic cell mediated activation of T cells

DCs have been called “nature’s adjuvant” due to their ability to coordinate many protective immune functions in response to infections. DCs have the unique capacity to stimulate naïve T cells and are thereby essential for the induction of primary immune responses (Figure 5). The high capacity of DCs, compared to other antigen-presenting cells, to induce T cell proliferation was first demonstrated in primary mixed lymphocytes reactions (MLRs) (109-111). DCs were shown to be approximately 100 times more effective at inducing T cell proliferation than macrophages and B cells (109). Also, the removal of DCs dramatically reduced the MLR stimulatory capacity of splenocytes (112).

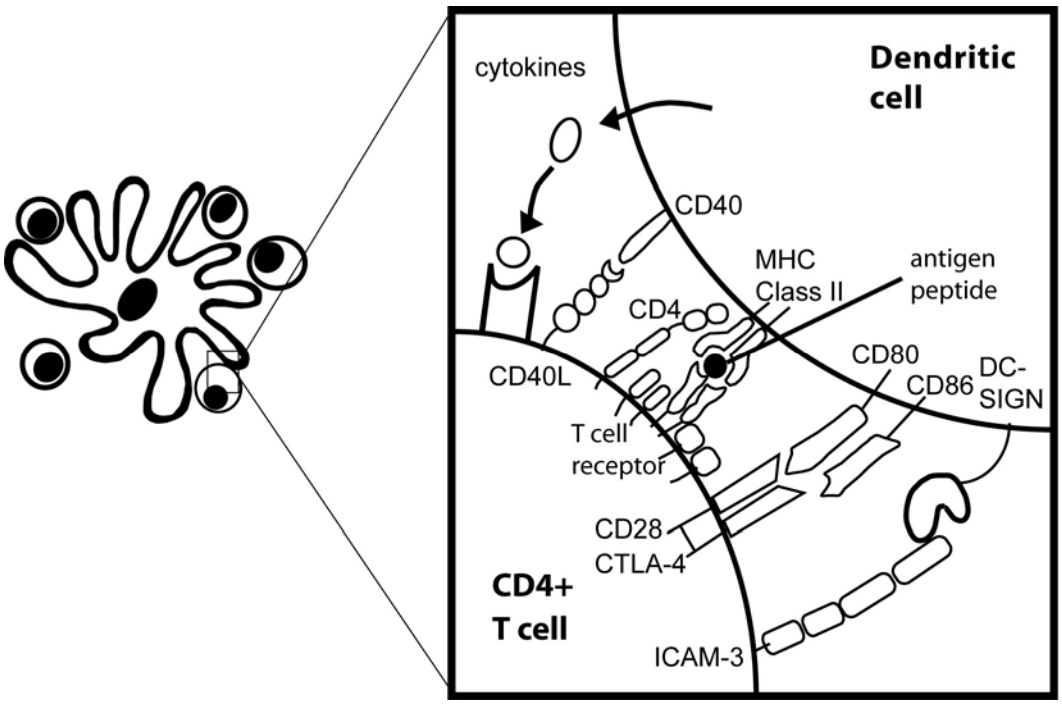

Figure 5. *The close interaction between a DC and a CD4+ T cell. The T cell receptor on the CD4+ T cell recognizes the peptide-MHC class II complex presented by the DC. CD40-CD40L interaction conditions the DC to produce cytokines and upregulate the expression of the co-stimulatory molecules CD80/CD86. In turn, CD80 and CD86 bind to CD28 or CTLA-4 on the T cell. Binding of co-stimulatory molecules to CD28 provides a signal for T cell activation, while CTLA-4 engagement downregulates T cell activity.*

DCs efficiently cluster with T cells (111, 113), which is probably a vital feature for efficient induction of T cell responses. In line with their higher expression of MHC and co-stimulatory molecules, mature DCs are superior to immature DCs at inducing T

cell proliferation (28). Furthermore, mature DCs can rapidly polarize immune responses with different effector functions, depending on the cytokines they secrete (114, 115). Cytokines are small proteins or glycoproteins that often act locally and regulate the immune system in a paracrine or autocrine fashion. If DCs secrete IL-12, IL-18 and RANTES the T cell response is skewed to a type 1 (Th1) response where the CD4+ T cells subsequently produce IL-2, IFNγ and TNF, and provide help for a cellular cytotoxic CD8+ T cell response. Th1 responses are also characterized by the induction of antibodies of certain isotypes, mainly IgG2a (116). IL-12 has proven to be a key cytokine for successful activation of cytotoxic T cell responses against viral infections (117-119). In addition to IL-12, the interaction between CD40L and CD40 is critical to bring DCs to a state of maturation to efficiently induce T cell killer responses (120-122). On the other hand, DCs can be triggered to induce a type 2 (Th2) response, where the CD4+ T cell produce IL-4, IL-5, IL-6 and IL-13 and provide help to induce a humoral antibody response (114). The presence of IgE is often a reflection of a Th2 immune response. DCs can also activate other innate protective cells like natural killer (NK) and NKT cells (123, 124). In addition to their central role in innate and adaptive immunity, DCs can also induce tolerance. While the immuno-stimulatory functions require mature DCs, an increasing literature show that DCs that are not fully mature induce T cell tolerance and can dampen the immune response and protect the host from autoreactivity (125).

Given their functional characteristics, DCs can play a role in disease progression, not only by presenting the pathogen to the immune system and orchestrating the T cell immune responses, but also by spreading the infection throughout the body.

HIV-1

In 1981 the first reports were published of an increased incidence of opportunistic infections like *Pneumocystis Carinii* and *Candida albicans* as well as an aggressive form of Karposi´s sarcoma among sexually active, young gay men in the United States (126, 127). Many of these patients had low numbers of CD4+ T cells, which was believed to account for the immunodeficiency symptoms they presented. The disease was named acquired immunodeficiency syndrome (AIDS). Two years later,

1983, a retrovirus was isolated from patients with AIDS and proposed to be the causative agent for the disease (128, 129). Dr. Robert C. Gallo, at the time working at the National Cancer Institute, Bethesda, MD, argued that the retrovirus was the known human T cell leukemia virus I (HTLV-I), while the group of Dr. Luc Montagnier at the Pasteur Institute in Paris, stated that this virus was a new HTLV. The following year Gallo and co-workers published four papers in Science describing a virus related to HTLV-I and HTLV-II as the causative agent for AIDS and named it HTLV-III (130-133). Later the virus was renamed HIV-1 (134). When some laboratory workers were tragically infected with HIV-1, and later developed disease, it provided the final evidence that HIV-1 is the causative agent for AIDS (135). The identification of HIV-1 as the cause for AIDS led to a long debate between the scientists involved in this discovery. Recently, Gallo and Montagnier, published their personal views on the now historical events concerning the discovery of HIV-1 (136, 137).

The origin of HIV-1

Strong evidence shows that HIV-1 was transferred to humans from chimpanzees, which harbor the related SIVcpz. In addition, phylogenetic data show that the related HIV-2 originates from SIVsm in sooty mangabeys (138-141). These monkeys live in Central Africa and coastal West Africa. In these areas, nonhuman primates live in close contact with humans as pets and wild animals are hunted and used as a source of food, which enables routes of cross-species transmission (142). Retrospectively, the earliest documented case of HIV-1 infection in humans was identified in a plasma sample from 1959, collected in former Leopoldville, Belgian Congo, now Kinshasa, Democratic Republic of Congo (143). Most likely, SIV was transmitted to humans by cutaneous or membrane exposure to infected animal blood (138). Based on phylogenetic analyses, it was established that these zoonotic transfers probably occurred repeatedly over time, no fewer than seven times and possibly more (138, 144-146). A broad range of factors have been suggested to explain the appearance of AIDS as an epidemic in the 20th century, including social disruption, urbanization, prostitution, population growth and additional changes in social behavior that are not yet fully understood (147, 148).

The HIV-1 structure and life cycle

HIV-1 is classified as a member of the Retroviridae family in the Lentivirus genus. The HIV-1 genome consists of two positive single-stranded RNA copies of approximately 9.2 kb and is composed of three major structural genes; *gag*, *pol* and *env*. HIV-1 *gag* codes for the nucleocapsid, capsid and matrix proteins (p6, p7, p17 and p24). HIV-1 *pol* codes for reverse transcriptase (RT), integrase and protease, and HIV-1 *env* codes for the envelope glycoproteins (gp120 and gp41). The HIV-1 provirus contains six additional open reading frames encoding the regulatory proteins Tat and Rev, and the accessory proteins Nef, Vif, Vpr and Vpu (149, 150).

HIV-1 infection is initiated when the virus binds via gp120 to the CD4 receptor of the target cell (151, 152) (Figure 6). After binding, the virus fuses with the membrane by interaction with gp41, gp120 and a co-receptor on the target cell, usually chemokine receptor CCR5 or CXCR4 (153-156).

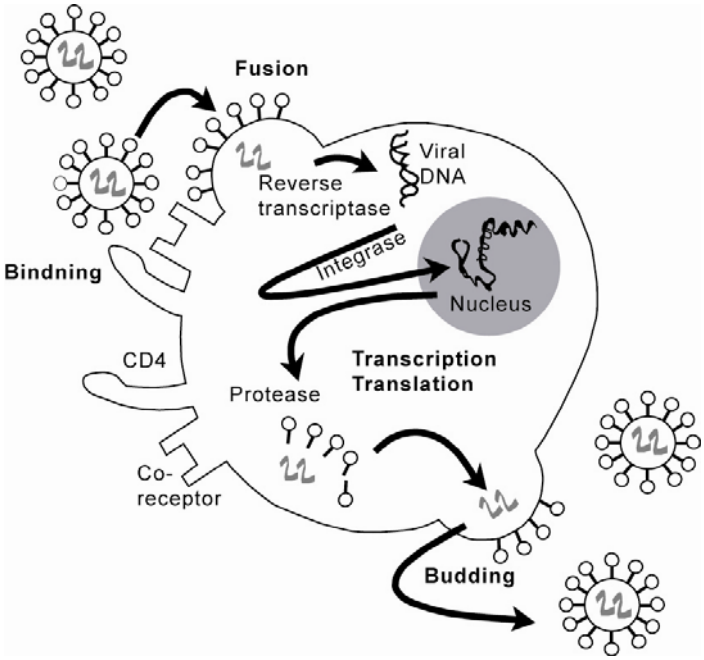

Figure 6. *The HIV-1 life cycle. The virus infects a susceptible cell by binding to CD4 and a co-receptor and fusing with the cell membrane. The viral genome is reverse transcribed and integrated in the host genome. After transcription and translation of the viral genome, new viral particles are assembled. Finally, mature infectious virions bud off from the infected cell.*

HIV-1 isolates that utilize CCR5 to enter the target cell are referred to as R5-using isolates or R5 isolates, while isolates that use CXCR4 in short are called X4 isolates. After fusing, the virus enters the cell cytoplasm and the viral RNA genome is reverse transcribed by RT to obtain a double stranded DNA copy, which is transported into the cell nucleus (157). The proviral DNA is stably integrated in the target cell genome by the viral enzyme integrase. The virus can remain dormant in this stage for long periods of time, before it is activated and transcribed; replicates, assembles and

eventually buds from the cell membrane. There is an apparent preference for CCR5-using isolates in the establishment of primary infection in most individuals (158-160), although the tropism broadens as a result of mutations in the V3 loop of the virus envelope protein, allowing the usage of a wider repertoire of co-receptors (Figure 7). When the virus quasispecies (the population of virus variants that have evolved in the host from the original infecting strain) change the overall usage of co-receptor from CCR5 to CXCR4, there is usually a sharp decline in the number of CD4+ T cells as well as increased viral load (161).

The course of HIV-1 infection

In general, HIV-1 infection follows three phases (Figure 7); the acute or primary infection within the first weeks of infection can be asymptomatic or manifest as mononucleosis-like illness. The level of virus in the plasma peaks and gradually declines to reach a steady state level, also called the viral set point.

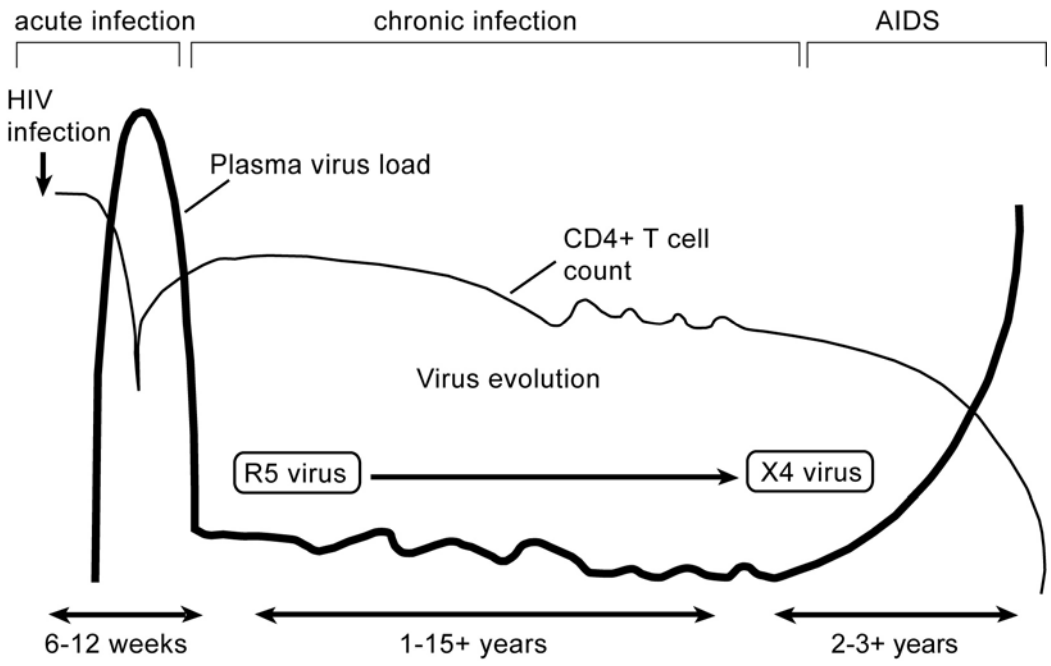

Figure 7. *Diagram of the course of a typical HIV-1 infection.*

The drop in viral load coincides with the appearance of HIV-1 specific cytotoxic T lymphocytes (CTLs, (162)). The chronic infection that follows can be clinically asymptomatic for many years. However, ultimately the immune system fails to control the infection, and as the disease progresses there is a decrease in CD4+ T cells and

a decline in the function of both innate and adaptive immune mechanisms (163, 164). This makes the patient susceptible to opportunistic infections and results in the third and final stage of HIV-1 infection: AIDS (165-167). WHO/UNAIDS estimates that 38 million people are currently infected with HIV-1. 4.8 million people were newly infected with HIV-1 in 2003 and 2.9 million died of AIDS during 2003 (168).

A hallmark of HIV-1 infection is the loss of CD4+ T cells. T cell dynamics are affected by HIV-1 infection, and the lifespan of both CD4+ and CD8+ T cells is shortened in HIV-1 infection to about one third of the lifespan of T cells in a healthy individual (169). HIV-1 replicates most efficiently in activated T cells (170), which could explain the qualitative loss of CD4+ T cell help, both to HIV-1 itself but also to other recall antigens (171). Also, HIV-1 preferentially infects HIV-1 specific CD4+ T cells (172). One feature of HIV-1 pathogenesis appears to be the failure to replace lost CD4+ T cells, which could be explained by thymic impairment (173). In addition, impaired DC function or generalized immune activation may also influence the loss of CD4+ T cell function and the course of HIV disease progression.

Dendritic cells in HIV-1 infection

Since HIV-1 infection is characterized by loss of CD4+ T cells and failure to control the infection, eventually rendering the patient susceptible to opportunistic infections, one could argue that exclusively T cells are of interest to study in this disease. However, as efforts are being made to understand the pathogenesis of the disease, the number of factors that contribute to disease presentation increase. One contributing factor could be defects in DCs, impacting on the initiation of T cell activation and subsequent T cell responses ((174-179), **paper I**). DCs express the receptors required for HIV-1 infection; CD4 and the most commonly used co-receptors CCR5 and CXCR4 ((180-182), **papers I-II**). MDDCs and dermal DCs also express a C-type lectin called DC-SIGN that has been shown to facilitate transfer of HIV-1 from DCs to T cells in vitro (70). Also, in vivo DCs are located at sites where initial transmission of HIV-1 is most likely to occur, like mucosal surfaces and blood. Therefore, DCs could serve as one of the initial target cells of HIV-1 infection, and

contribute to the overall immunodeficiency observed in HIV-1 infected individuals. Hence, it is important to study DCs in the context of HIV-1 infection.

HIV-1 infection of dendritic cells

The question of whether DCs are infected with HIV has been a matter of debate for several years. It has been difficult to answer that question mainly for three reasons: i) DCs represent a relatively rare cell population in tissue; ii) DCs migrate from peripheral tissue upon contact with antigen. Conclusively, infected DCs remain in the periphery less than 2-3 days following mucosal inoculation of the virus (183, 184). Finally, iii) HIV-1 infection in DCs has been difficult to detect because viral replication in DCs occurs at much lower levels than in CD4+ T cells, that have been studied in detail with respect to HIV-1 infection. Despite the difficulties mentioned, the data obtained to date suggest that DCs are susceptible to HIV-1 infection and may represent a cellular reservoir for HIV-1 in infected individuals. In 1987, LCs from HIV-1 infected individuals were reported to contain HIV-1 as determined by staining for HIV proteins in explant cultures of skin biopsies (185, 186). Later both MDCs and PDCs have been identified as cellular reservoirs for HIV-1, in addition to LCs, albeit to a limited extent (187-190). However, some data state that blood DCs do not constitute an HIV-1 reservoir in vivo (191). Also spleen DCs are reservoirs for HIV-1 in infected individuals (192). In addition, a number of reports have confirmed the susceptibility of DCs to HIV-1 infection in vitro (35, 193-199).

Data suggest that once HIV-1 infection has established, the predominant source of new virus production and the main reservoir of HIV-1 is CD4+ T cells (183, 184). DCs may have a greater role in retaining the virus and spreading it to the more permissive CD4+ T cells, than producing large amounts of virus themselves.

In addition to DCs and CD4+ T cells, HIV-1 has been found to infect several cell types of the immune system (200), like macrophages (201, 202), CD8+ T cells (203), NKT cells (204, 205) as well as NK cells (206). However, it is not fully understood if and to what extent HIV-1 infection of these cells contributes to the pathogenesis of the disease.

Loss of dendritic cells during HIV-1 infection

Several studies have shown that the number of circulating MDCs and PDCs is reduced in HIV-1 infected individuals (176, 189, 207-213). Also, the loss of circulating DCs correlates with an increase in HIV-1 viral load (211, 212, 214). A recent report indicates a correlation between the recovery of circulating PDCs during ART and a lower viral load rebound after treatment interruption in patients with primary HIV-1 infection (215). It remains unclear why DCs are lost during HIV-1 infection. Several hypotheses have been proposed to explain this phenomenon; i) failure of DC precursors to differentiate into characteristic DCs, ii) death of DCs due to HIV-1 infection and iii) relocalization of DCs to secondary lymphoid tissues, perhaps as a consequence of DC maturation induced by the virus or induced when DCs capture HIV-1 for antigen processing and presentation. During acute HIV-1 infection, an increased frequency of DCs is detected in lymph nodes, suggesting that migration from the periphery to these sites is increased (174). However, patients with AIDS have a reduced number of DCs in their lymph nodes, indicating that redistribution does not fully explain the loss of DCs in HIV-1 infection (174).

Functional impairment of DCs in HIV-1 infection

Viruses can suppress DC function in a number of ways, including induction of apoptosis, inhibition of maturation, impaired cytokine production, migratory deficiencies and inhibition of T cell activation. DCs isolated from HIV-1 infected individuals have a reduced capacity to stimulate resting T cells in vitro, compared to DCs isolated from HIV-1 seronegative persons (175-179). There is also a defect in IL-12 production in PBMCs isolated from AIDS patients (216, 217). The observed IL-12 impairment could be overcome by addition of soluble CD40L to monocyte cultures from HIV-infected patients (218). DCs isolated from HIV+ individuals produced similar amounts of IL-12 following stimulation with CD40L, as DC from HIV negative individuals (219). Both the nature of stimulus and the stage of disease determine the amount of IL-12 secreted from PBMCs isolated from HIV-1 infected individuals (220). In addition, there is an accumulation of DCs in the lymphoid tissue of HIV-1 infected patients during acute infection. These DCs have a reduced expression of the co-stimulatory molecules CD80 and CD86, which may limit their capacity to generate HIV-1 specific T cell responses (174). This reduced expression of co-stimulatory molecules could be a direct consequence of the virus or an indirect effect of the

infection, causing insufficient stimulation of DCs. An indirect impairment of DC function could be that DCs do not get appropriate stimulation and subsequently can not provide sufficient stimulation themselves to initiate proper immune responses. Increasing evidence suggests that HIV-1 has evolved mechanisms to suppress CD40L expression (221, 222). The lack of efficient immune responses against HIV-1 in infected individuals could therefore in part be explained by the lack of CD40L, as the interaction between CD40L and CD40 on DCs is crucial for maturing DCs in a way sufficient to trigger CTL responses (120-122). Furthermore, dysregulation of cytokine production in DCs could also contribute to incomplete CD4+ and CD8+ T cell activation. The failure of specific T cell help together with DC dysfunction might augment the disability of the host to respond to emerging virus quasispecies and to mount appropriate HIV-1 specific CTLs (223).

Treatment strategies for HIV/AIDS

At present there is no cure for HIV-1. The need to develop new therapies or a vaccine for HIV/AIDS is urgent, as the number of people infected with HIV-1 has grown to 38 million and continues to increase at an alarming rate in many developing countries. However, the existence of HIV-1 infected individuals that are able to control their infection as well as people that are repeatedly exposed to HIV-1 but not infected, provide hope for the possibility to find ways to prevent infection. Extensive efforts are made to gain a better understanding of the virus, the immune responses that contain HIV-1 infection, and the complex mechanisms used by the virus to evade those immune responses. Since HIV-1 targets, infects and compromises cells of the immune system, the interactions between this virus and the host are both complex and offer insights into mechanisms of immune defences.

Current treatment of HIV-1 infection

The introduction of triple antiretroviral treatment (ART) in 1996 dramatically reduced the rate of disease progression and death through control of viral replication and partial reconstitution of the immune system (224-226). However, ART alone does not seem to result in improved HIV-1 specific immune function (225, 227, 228). Most of

the approved antiretroviral drugs currently in use are inhibitors of viral enzymes with no known direct human analogues; the viral RT and protease. These enzymes are essential for the virus replication and interference with them inhibits production of intact and infectious virus particles. In addition, one fusion inhibitor, T-20 is currently approved for clinical use in Sweden.

Initially, hopes were high that the virus could be controlled and eradicated, and therapy eventually stopped (229). However, in most chronically infected patients, attempts to discontinue ART have resulted in a rapid increase in plasma viral load and a decrease in CD4 counts (230, 231). Supervised or structured treatment interruptions (STI) has been considered as an approach to provide limited, but hopefully sufficient antigenic stimulus to the new naïve T cells that are generated during ART and to educate these cells to target HIV-1 (232). Indeed, STI in a limited number of patients with acute infection resulted in immune control (233), but unfortunately these data have been difficult to reproduce especially in patients with chronic HIV-1 infection (230). The probable cause of failure in STI, so far, is the persistence of a long-lived reservoir of infected cells, which consequently results in the need for lifelong treatment of HIV-1 infected patients (234).

Although the introduction of ART has changed the clinical course of HIV-1 and AIDS in a radical way, there are still drawbacks to consider. One of the main difficulties in a global perspective is poor access to antiretroviral drugs. The effective treatment is both costly and requires an infrastructure that makes it unavailable for most people in developing countries. The major problem in treated patients is compliance, where inconsistent intake of the drugs results in increased risk for development of drug resistant virus strains. Also, the therapy is accompanied by severe side effects like diarrhea, hyperlipidemia, muscular and neurological toxicity and increased risk of diabetes, hypertension and cardiac infarcts (235, 236). Therefore, the ideal solution to contain or even eradicate the virus and the AIDS epidemic would be to develop a vaccine. New and exciting strategies are being exploited to find prophylactic or therapeutic vaccines and improve antiretroviral treatment.

Introduction to vaccines

Vaccines against infectious diseases have had great impact on human health world wide, as they prevent disease as well as disease symptoms of global infections. Two major categories of vaccination exist: passive and active immunization. In brief, passive immunization depends on the administration of purified antibodies or antibody containing sera against a certain pathogen, to an individual at risk of contracting the particular infection. Passive immunization results in temporal protection against infectious agents, for example in newborns who receive maternal antibodies via breastfeeding. Active immunization, on the other hand, stimulates the immune system to induce effector molecules and/or effector cells against the immunogen. Edward Jenner noted that milkmaids who had contracted cowpox were immune to subsequent smallpox infection. Jenner confirmed this by injecting an eight-year-old boy with material from cowpox lesions and later deliberately infected the boy with smallpox. The boy was immune to smallpox and did not develop the disease (237). Later, Louis Pasteur observed that chickens inoculated with old cholera cultures caught the disease but recovered and developed immunity to subsequent cholera infection, while chickens inoculated with fresh cholera cultures died (238). Pasteur argued that the cholera had weakened by long-term culture and called the attenuated cholera strain a vaccine. In line with these findings, first-generation vaccines were live, attenuated pathogens, a strategy with obvious safety concerns. Next, chemically or physically inactivated pathogens were developed for immunization. Third-generation vaccines were purified or synthetic proteins derived from the pathogen and lately, a fourth-generation vaccine strategy is developed based on DNA and virus vectors coding for immunogenic proteins (239). Prophylactic vaccination prevents infection by inducing specific memory and effector immune responses that provide sterilizing immunity in non-infected individuals. Therapeutic vaccination, in stead prevent severe complications in a chronically infected individual by reinforcing and/or broadening specific immune responses in the host (240). The development a prophylactic HIV-1 vaccine, which could be administrated to people on a large scale, will hopefully be a reality. However, maybe it is more likely to first find a therapeutic vaccine that could improve the conditions for already infected individuals.

Requirements on an HIV-1 vaccine

In theory, to generate efficient protection against HIV-1, an ideal vaccine should stimulate four different components of the immune system. It should generate broadly cross-reactive neutralizing antibodies, induce high levels of antiviral T cells, stimulate mucosal immunity as well as provoke the innate immune system (164, 241). To obtain protective immunity, the major targets for an HIV-1 vaccine are most likely steps involved in virus entry and virus replication (242). Antibodies that block entry of the virus to the target cell could neutralize the virus and prevent infection. However, it has proven very difficult to generate antibodies that can neutralize primary isolates of HIV-1 (243). Also, the high error rate of the viral enzyme RT generates large antigenic variation in the viral proteins gp120 and gp41 involved in cell surface binding and cell entry (244). An effective vaccine should probably also induce cellular immune responses, which are mediated by T cells. Given the difficulty to raise neutralizing antibodies against HIV-1, most current vaccine strategies aim to induce HIV-1 specific T cell responses. Vaccines based on induction of HIV-1 specific T cell responses might not prevent infection, but can control virus replication. Still, HIV-1 specific cytotoxic T cells have been found in repeatedly HIV-1 exposed but uninfected individuals (245), providing hope for a protective capacity of T cell response induced by vaccination. CD8+ T cells clear infections by lysing infected cells (246), by release of antiviral cytokines (247-249) and chemokines (248, 250, 251). Induction of HIV-1 specific T cell helper responses is most certainly required for long-term control, since these cells are needed to maintain CTL function (252). However, there are still little data defining the magnitude, breadth and specificity of CD4+ helper or CD8+ CTL responses that results in true clinical benefit (253-255). The challenges in creating an effective HIV-1 vaccine are closely linked to the characteristics of the virus itself. In addition to variations within an individual patient there are at least 12 subtypes of the virus with broad geographical distributions (256). This results in difficulties in generating a worldwide applicable vaccine. Finally, since HIV-1 is a retrovirus, it integrates in the host genome and can hide from the immune system (257), evidently for long periods of time.

No single immune mechanism has been found to unaccompanied be prognostic for either protection against HIV-1 infection, viral clearance or disease progression. Historically, most vaccine candidates have entered clinical trials with limited

knowledge of their ability to stimulate immune responses and what immune responses result in protection against the pathogen (239). However, most effective vaccines available today are directed against acute virus infections like smallpox, polio, measles, rubella and influenza and protection is associated with generation of virus-specific neutralizing antibodies (258, 259). Cell-mediated immune responses are important in the control of established chronic infections like CMV and Epstein-Barr virus (EBV) (260, 261). In experimental SIV infection, vaccine-induced cellular immunity has been shown to control chronic disease (262-266). An increased knowledge in immunology allows us to obtain a more detailed understanding of the protective effects of vaccines and the correlates to protection.

Dendritic cells in HIV-1 immunotherapy

DCs are essential to the initiation of T cell responses against foreign antigens and probably play a role in HIV-1 pathogenesis. The administration of cytokines like IL-2 and GM-CSF (267-270) aim at modulating DCs and subsequently T cell responses in vivo. Other strategies considered are administration of factors that stimulate DC activity and mount stronger immune responses in HIV-1 infected individuals, like soluble CD40L or TLR ligands (271). As DCs express TLRs, and signaling via TLRs can affect the ability of DCs to orchestrate adaptive immunity, the effects of TLR ligation bridge innate and adaptive immunity (272, 273). TLRs could be useful drug targets, since they are prime sensors of microbial products. Thus, the interest in using compounds that bind to TLRs has intensified as these molecules could be used as vaccine adjuvants (43). In addition, therapeutic strategies are developed to generate or enhance HIV-1 specific cellular immunity by targeting DCs in vivo or using DCs loaded with antigens. Several studies have reported attempts to develop HIV-1 antigen containing vectors that target DCs (274-276). In animal models, immunization with DCs loaded with peptides or viral vectors that target DCs induces immune responses against viruses (277). In 2003, Lu and colleagues published the first evidence in an animal model that therapeutic immunization can augment immune control of SIV (278). Rhesus macaques with an established SIV infection were immunized five times with autologous DCs loaded with chemically inactivated SIV in two week intervals. This resulted in a marked reduction of viral load and an increase in CD4 counts, all in the absence of ART. The animals were immunized early in the disease progression, 56 days post infection. As mentioned earlier,

intervention by STI in acute infection has been promising also in humans, but less successful in chronic infection (230, 233). Thus, although the data from Lu and co-workers suggest that therapeutic vaccines work, they need to be repeated in animals with a chronic infection. Also, preparation of autologous DCs for in vitro loading and readministration is perhaps not feasible due to manufacturing requirements as an ideal HIV-1 vaccine.

Dendritic cell immunotherapy using apoptotic cells

There are several ways in which DCs can capture viral antigens, besides direct infection, and induce T cell responses. DCs can present nonreplicating virus, which are abundantly found in the plasma of HIV-1 infected individuals (279). DCs can also efficiently take up and present immune-complexed antigens (101, 280). In addition, DCs can present antigens derived from apoptotic cells. In vitro studies have shown that uptake of apoptotic virus-infected cells by DCs results in efficient presentation of viral epitopes on MHC class I (281-283).

Apoptosis, or programmed cell death, is a process of great importance during development and maintenance of tissue homeostasis. The process is characterized by caspase activation, DNA fragmentation and formation of apoptotic bodies (284). These apoptotic bodies are membrane-enclosed vesicles that contain both proteins and DNA from the cell of origin. The apoptotic bodies formed upon cell death are cleared through uptake by phagocytosing cells and do not induce immune responses, in contrast to another type of cell death; necrosis, which is usually the cause of inflammation and tissue damage (285). Still, in vitro studies have shown that uptake of virus-infected apoptotic cells by DCs result in presentation of viral antigens and induction of immune responses (274, 281-283, 286). Matzinger hypothesized that apoptosis of virus-infected or otherwise damaged cells may provide a signal that alerts the immune system (287, 288). Such signals could be inflammatory cytokines, necrotic cells, bacterial components or double-stranded RNA, which induce maturation of DC, a crucial step in the initiation of immune responses (285, 289-291). It has previously been demonstrated that EBV, HIV-1 and oncogenic DNA present in apoptotic cells can be transferred to antigen-presenting cells, analogous to the horizontal gene transfer frequently used as a mechanism to exchange genetic information in bacteria (292-294). In these studies, the transferred DNA was

subsequently expressed in the recipient antigen-presenting cell. The efficiency of horizontal DNA transfer correlated with the phagocytosing capacity of the recipient cells. We propose that horizontal gene transfer after uptake of apoptotic HIV-1 infected cells could be the hypothetical base for a novel HIV-1 vaccine strategy, where autologous apoptotic HIV-1 infected cells are administrated. This strategy has some theoretical advantages when considering the above-mentioned difficulties in constructing an HIV-1 vaccine. Using the patients' own cells to generate apoptotic cells would include many or all of the virus variants present in the patient and potentially overcome the problem with high variability of HIV-1. Apoptotic cells contain both DNA and proteins, a combination shown to be a strong inducer of immune responses in vaccination trials (295, 296). Apoptotic HIV-1 infected cells also include the entire HIV-1 genome and given the high diversity of HIV-1 and concomitant escape from immune control (297), it is probably advantageous to include as many genes as possible to elicit a broad immune response.

Taken together, an increased understanding of the immunobiology of DCs in health and disease could help us exploit the features of DCs and use them to design effective immunotherapy.

Methods

The methods used in the studies included in this thesis are described in the original papers (**papers I-IV**). However, some of the central methods and experimental set-ups used in the different studies are outlined below.

Generation of monocyte-derived dendritic cells

Monocyte-derived DCs (MDDCs) were differentiated from PBMCs as described earlier (26, 298). PBMCs were separated from healthy blood donors using density gradient and monocytes were allowed to adhere for 1.5 hours at 37°C. Cells were carefully washed 3 times and adherent monocytes were cultured for 6 days in medium supplemented with 10% fetal bovine serum (FBS) and recombinant human IL-4 and GM-CSF, to obtain immature MDDCs (Figure 8). The frequency of MDDCs ranged from 75-90% in the cell cultures on day six. Immature MDDCs were CD14- CD1a+ with low expression of CD40, CD80, CD83 and CD86.

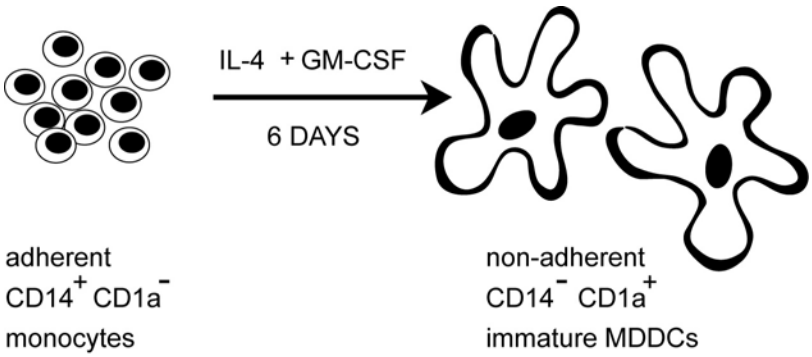

Figure 8.
In vitro generation of MDDCs. Adherent CD14+ CD1a- monocytes isolated from PBMCs were cultured in the presence of human recombinant IL-4 and GM-CSF for six days and developed into immature non-adherent CD14- CD1a+ MDDCs.

The frequency of $CD14^+$ cells was 5-10% while the frequency of contaminating $CD3^+$ cells ranged from 5-15% in the culture on day 6. Although monocytes can be enriched for based on their capacity to adhere to plastic, the cells are only loosely adherent and it is difficult to obtain cultures free of other cell populations. In some experiments, it was essential to obtain MDDCs absolutely free of contaminating cells and thus, CD14+ monocytes were enriched from PBMCs by negative selection. Monocytes were cultured in the presence of IL-4 and GM-CSF, as described. After 6 days 95% CD1a+ immature MDDCs were obtained with low to undetectable levels (<0.6%) of CD3+ T cells and CD14+ monocytes.

Isolation of circulating blood myeloid and plasmacytoid dendritic cells

Recently, we established a direct isolation procedure to purify relatively high numbers of primary human CD123+ PDCs and CD11c+ MDCs from blood (43). Briefly, PBMCs were collected from healthy HIV-1 seronegative blood donors by automated leukapheresis. Enriched populations of lymphocytes and monocytes were obtained by counterflow centrifugal elutriation. MDCs and PDCs were isolated from elutriated monocytes using magnetic beads followed by sequential separation on AutoMacs (Miltenyi Biotec), (Figure 9). The BDCA-4 and CD1c isolation kits were used for isolation of PDCs and MDCs, respectively.

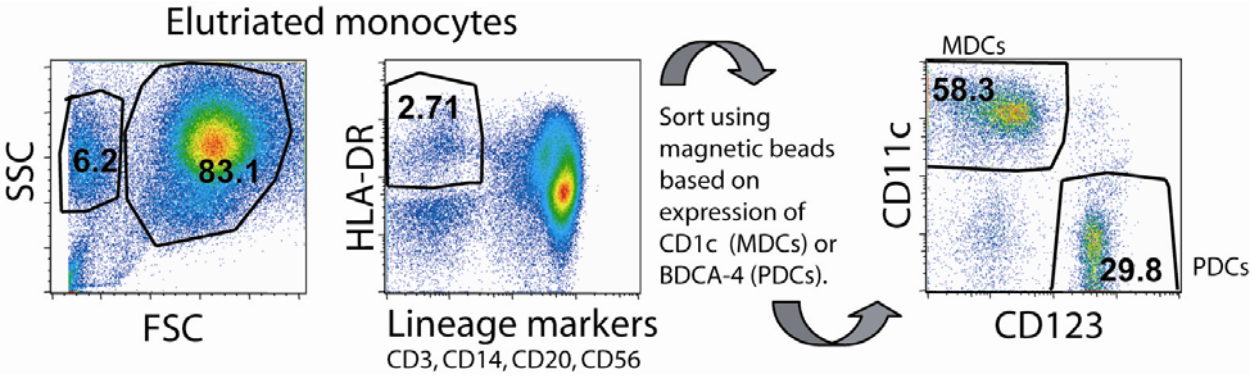

Figure 9. *Isolation of human blood MDCs and PDCs. CD11c+ MDCs and CD123+ PDCs are isolated from elutriated monocytes, which are mainly large CD14+ cells. In the elutriated monocyte fraction, the DC subsets are characterized by high expression of HLA-DR and lack of expression of the lineage markers CD3, CD14, CD20 and CD56. MDCs are positively selected based in their expression of CD1c and PDCs are isolated based on their expression of BDCA-4 using magnetic beads.*

To maintain viability, the PDCs and MDCs were cultured in complete medium supplemented with 10% FBS and the recombinant human cytokines IL-3 or GM-CSF, respectively. On average, the recovery of isolated DCs was $1.1x10^6$ PDCs and $2.2x10^6$ MDCs per 10^8 elutriated monocytes. The subsets of DCs were highly enriched (>90%) as determined by lack of the lineage markers (CD3, CD20, CD14 and CD56), and expression of HLA-DR and CD123 or CD11c. The contaminating cells were CD14+ monocytes. Overnight culture in IL-3 or GM-CSF of freshly sorted PDCs and MDCs, respectively, lead to development of characteristic DC morphology (especially MDCs) and immature DC phenotype with low expression of CD40, CD80, CD83 and CD86.

Dendritic cell:CD4+ T cell co-cultures

DCs and CD4+ T cells were isolated from elutriated monocytes and lymphocytes, respectively, from healthy CMV seropositive donors with a detectable CD4+ T cell recall response against CMV. MDCs and PDCs were isolated as described above. Negatively isolated CD4+ T cells (memory and naïve) were obtained by depletion of CD1c+, CD8+, CD14+, CD15+, CD19+, CD56+ and BDCA-4+ cells using magnetic beads and separation on AutoMACS and frozen until further used. After overnight incubation, the MDCs and PDCs were infected with HIV-1_{BaL} and HIV-1_{IIIB} or mock and the cells were cultured for 72 hours. In some experiments, azidothymidine (AZT) was added to the cultures prior to addition of the virus isolate to prevent HIV-1 infection. The DCs were exposed to HIV-1 for 72 hours and in some cases stimulated with the TLR7/8-ligand R-848 during the final 24 hours, to induce DC maturation. Thereafter, HIV-1 exposed or unexposed DCs were thoroughly washed in pre-warmed media to minimize transfer of residual non-cell associated virus and then co-cultured with sorted autologous CFSE-labeled CD4+ T cells in a DC:T cell ratio of 1:10 (Figure 10). Most co-cultures were stimulated with whole CMV antigen lysate to activate CMV-specific memory T cells but in some experiments, the T cell receptor (TCR) superantigen SEB was used as an alternative antigen. The DC:T cell co-cultures were cultured for 1-8 days with addition of brefeldin A during the last 12 hours of co-culture. In some experiments, the protease inhibitor Indinavir was added to the cells at initiation of co-culture to prevent spreading and propagation of the virus among T cells, after the initial transfer of HIV-1 from DCs to T cells. After harvesting, the cells were surface stained with CD11c (for co-cultures including MDCs) or CD123 (for co-cultures including PDCs). Cells were then fixed and permeabilized for intracellular staining of HIV-1 p24 and cytokine expression (IFNγ, TNFα and IL-2). The cells were immediately analyzed to prevent leakage of CFSE dye from the cells. In addition, after 10 hours and 3.5 days of co-culture, four different fractions of T cells were sorted using a FACSVantage SE/DiVa (Becton Dickinson). Cells were gated on CD11c-/CD123- CFSE labeled T cells and subsequently 1) undivided cytokine producing, 2) dividing cytokine producing, 3) undivided cytokine non-producing and 4) dividing non-cytokine producing T cells were sorted. The T cells were subsequently analyzed for their content of HIV-1 sssDNA (an early product of replication) and full-length HIV-1 gag DNA copies per 10^5 cells using quantitative real time PCR.

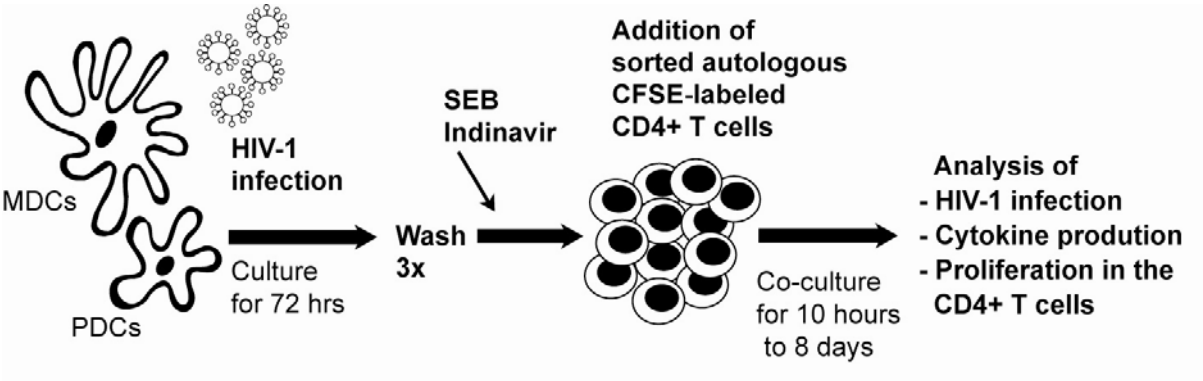

Figure 10. *Co-culture of HIV-1 exposed DCs and autologous CD4+ T cells. MDCs, PDCs and CD4+ T cells were isolated from elutriated monocytes and lymphocytes, respectively, from healthy CMV seropositive donors with a detectable CD4 recall CMV response. Total CD4+ T cells were isolated and frozen until further used. After overnight incubation, the DCs were infected with HIV-1 and cultured for 72 hrs. Thereafter, DCs were thoroughly washed to minimize transfer of residual non-cell associated virus and then co-cultured with sorted autologous CFSE-labeled CD4+ T cells in a DC:T cell ratio of 1:10 in the presence of antigen (CMV or SEB) and in some cases Indinavir. The DC:T cell co-cultures were cultured for 10 hours to 8 days. Thereafter the cells were harvested, stained and analyzed by flow cytometry.*

Gating strategies in flow cytometric analyses of dendritic cell: T cell co-cultures

To obtain as much information as possible from the DC:T cell co-cultures, we set up a gating scheme to analyze both the DCs and the T cells in the same sample (Figure 11). First, DCs and T cells were separated based on cell size (FSC) and granularity (SSC), where DCs were found in the population of large and more granulated cells and T cells were less granulated and ranged in size from small (non-proliferating) to large (proliferating). Dead cells were excluded based on size as well as propidium iodide staining. DCs and T cells were further separated based on CFSE staining (as all T cells were dyed before initiation of co-culture) and CD11c (MDCs) or CD123 (PDCs). However, in the T cell gate, the highly CFSE expressing events, that had a brighter CFSE staining than the freshly stained T cells, were excluded as they most likely represent dead or dying cells and cellular conjugates (299). HIV-1 infection assessed by p24 expression as well as IFNγ, TNFα and IL-2 production could be

analyzed in both DCs and T cells. T cell proliferation could also be determined based on CFSE dilution.

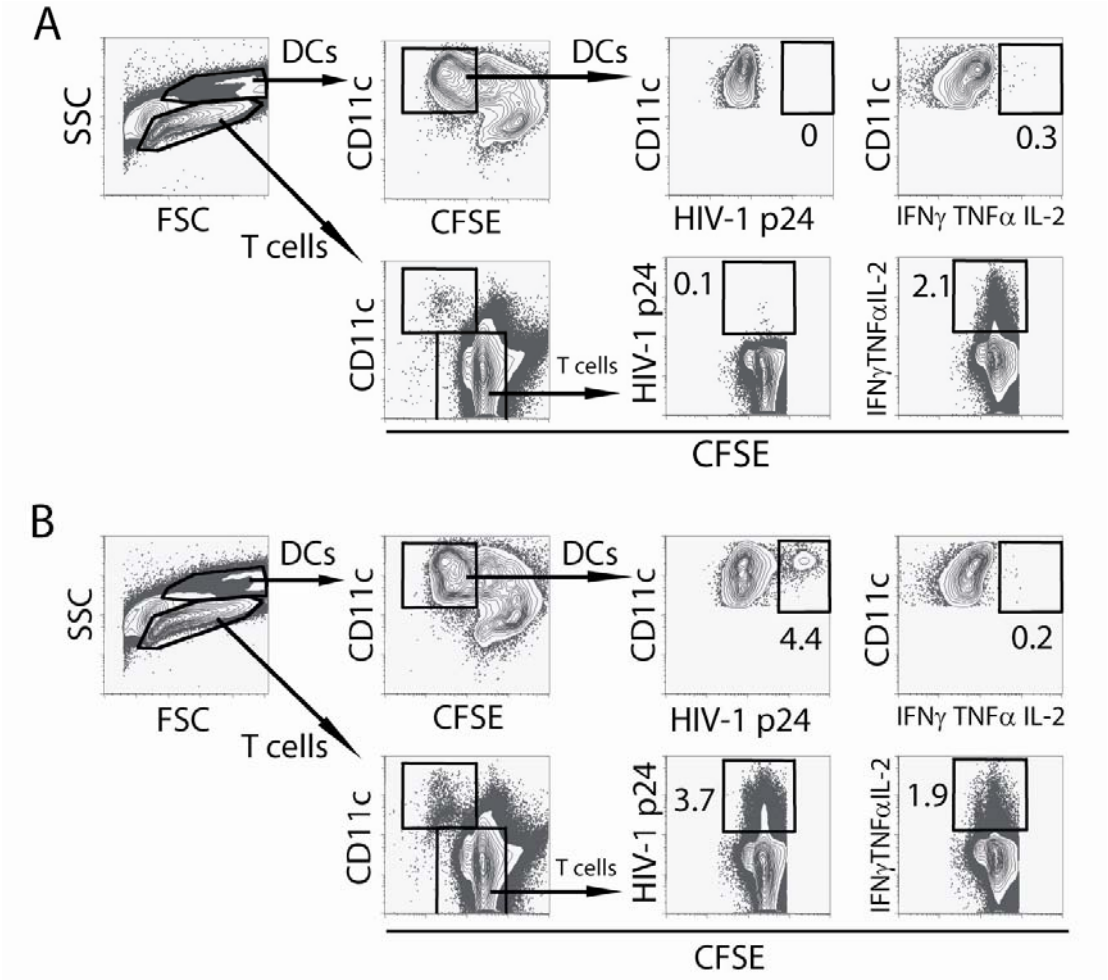

Figure 11. *Gating strategies for the DC:CD4+ T cell co-cultures. Unexposed (A) or HIV-1_{BaL} exposed (B) MDCs were co-cultured with autologous CFSE-labeled CD4+ T cells for 3.5 days.Tthe cells were subsequently harvested, stained and analyzed by flow cytometry. First, DCs and T cells were separated and dead cells were excluded based on cell size and granularity. DCs and T cells were further separated based on CFSE staining and CD11c (MDCs). The highly CFSE expressing events were excluded as they represent dead/dying cells or cellular conjugates. HIV-1 infection, cytokine production of the different cell populations and T cell proliferation could thereafter be determined.*

Results and discussion

Below I discuss the results obtained in the studies included in this thesis in a more general context. For detailed information on and graphic illustrations of the data, please see the included papers (**papers I-IV**).

Dendritic cells are susceptible to HIV-1 infection

An increasing number of reports show that DCs are susceptible to HIV-1 infection, both in vivo and in vitro (35, 187-190, 192, 194-199) However, the somewhat conflicting reports on the level of susceptibility to HIV-1 infection and the capability of DCs to support productive infection could be due to the diversity of techniques used for isolation and culture of DCs. In addition, infection protocols optimized for infection of CD4+ T cells have sometimes been applied to DCs, which often include long culture periods and stimulation conditions that are not appropriate for DCs. The methods to determine HIV-1 infection have mainly been based on detection of secreted viral proteins in the cell culture supernatant and/or detection of viral RNA or DNA. These methods commonly estimate the overall infection in a culture and do not allow for further studies of the HIV-1 infected DCs per se.

In vivo, DCs in mucosa and blood, where initial transmission of HIV-1 occurs, express an immature phenotype (Figure 4). Immature DCs are therefore more relevant to examine with respect to HIV-1 susceptibility. We established an infection protocol that allowed us to study early HIV-1 infection of immature DCs in vitro using intracellular staining against the HIV-1 protein p24 and flow cytometric analysis. We showed that immature MDDCs (**paper I**) as well as isolated primary immature PDCs and MDCs (**paper II**) were susceptible to HIV-1 infection in vitro and that the virus replicated in these DC subsets. We exposed DCs to purified and highly concentrated HIV-1_{BaL} (R5) and HIV-1_{IIIB} (X4) isolates with high multiplicity of infection to generate high and fast infection without inducing cell death. In contrast to CD4+ T cells that need stimulation to induce significant viral replication, HIV-1 infection of DCs does not require prior activation of the cells (196, 300). We found that MDDCs (**paper I**), MDCs and PDCs (**paper II**) replicated HIV-1 and expressed significant amounts of p24 intracellularly without prior activation. This allowed us to study early HIV-1

infection in DCs, as viral DNA transcripts could be detected already 3 hours after exposure and HIV-1 p24+ cells appeared after 24 hours of viral exposure and increased over time. No viral replication was detected when the DCs were cultured in the presence of AZT. AZT is a nucleoside analogue reverse RT inhibitor, which inhibits viral replication by hampering RT activity through competition with the natural deoxynucleotide triphosphates (dNTPs) for the enzyme-binding site or by incorporation into the growing DNA chain resulting in chain termination. Addition of AZT to the cell culture thus prevents productive infection by inhibition of reverse transcription of the viral genome, integration and subsequent formation of viral proteins and virus particles.

Interestingly, we found a difference in susceptibility of primary PDCs and MDCs to HIV-1_{BaL} and HIV-1_{IIIB} (**paper II**). We found that MDCs were more effectively infected by HIV-1_{BaL} than PDCs. MDCs were less susceptible to infection with HIV-1_{IIIB} than HIV-1_{BaL}. However, the differences in susceptibility of the DC subsets could not be correlated to the expression level (MFI) of the co-receptors CCR5 and CXCR4 on MDCs and PDCs prior to infection (**paper II**). Still MDCs express higher levels of CCR5 than PDCs, in analogy with their higher susceptibility to the R5-using HIV-1_{BaL}. Early after transmission, HIV-1 isolated from infected individuals is almost always CCR5-using (158-160). This could be due to the fact that CD4+ T cells and DCs in the mucosa and submucosa at the local site of infection express much more CCR5 than CXCR4 (301-303). MDCs, which express high levels of CCR5, are more prevalent in mucosal tissues, while PDCs normally are found only in blood and lymph nodes. Thus, MDCs may encounter HIV-1 in the periphery early after initial infection and contribute to the selection of virus strains that are transported to the lymph nodes. In contrast, PDCs may primarily be exposed to virus that reaches lymphatic tissues and may play a role in DC mediated transfer of HIV-1 to T cells during antigen presentation. One could thus speculate that the difference in susceptibility to HIV-1 infection between the DC subsets may play a role for the initial selection of virus at transmission as well as the virus spread to T cells.

Functional properties of HIV-1 infected dendritic cells

The effects of HIV-1 on DC function are poorly understood but most likely important to our understanding of the pathogenesis of the disease. The presence of detectable

p24+ DCs allowed us to extend prior findings with detailed studies of the effects of HIV-1 infection on DC maturation and cytokine production (**papers I-II**).

Phenotypic maturation

We found that in vitro HIV-1 exposure of MDDCs did not lead to detectable phenotypic maturation overall in the culture as determined by increased expression (MFI) of CD80, CD86, CD83 and HLA-DR compared to the unexposed donor-matched MDDCs (**paper I**), in agreement with previous reports (219, 304, 305). However, when we studied the expression of CD86 on the MDDCs that were not only exposed but HIV-1 infected (p24+), the average CD86 expression tended to be higher than on the uninfected p24- MDDCs in the same culture (**paper I**). HIV-1 exposure of primary MDCs and PDCs, using an identical infection protocol and virus dose as for MDDCs, resulted in a moderate increase in the expression of co-stimulatory molecules overall on MDCs (CD86) and PDCs (CD86, CD40 and CD83) after virus exposure compared to the medium control (**paper II**). It is therefore possible that i) HIV-1 infected DCs are more mature than uninfected DCs and ii) that primary DCs are more sensitive to HIV-1 exposure than in vitro-derived DCs. In vitro HIV-1 exposure of PDCs has previously been shown to induce significant upregulation of co-stimulatory molecules (199, 306). Although HIV-1 exposure alone did not induce maturation in MDCs, they matured as bystander cells to exposed PDCs (306). In our studies, regardless of prior differentiation caused by the virus, HIV-1 exposure and/or infection did not inhibit further maturation by CD40L or TLR ligation in any of the DC subsets. Furthermore, full maturation did not increase HIV-1 replication in infected DCs, which is a major concern when designing immunomodulatory therapies that target DCs. Taken together, regardless if HIV-1 exposure causes some differentiation or not, it at least appears that HIV-1 does not impair the ability of DCs to respond to stimuli and upregulate expression of essential co-stimulatory molecules. However, the presence of these receptors on the cell surface does not exclude that HIV-1 infected DCs could have an impaired antigen-presenting function.

As mentioned in the introduction, one theory as to why the frequency of circulating DCs decreases in HIV-1 infected individuals is that DCs become infected and die. We did not see an increased number of dead cells in either the MDDCs or the

primary DCs after 72 hours of HIV-1 exposure as compared to the medium control (**papers I-II**). However, at later time points (> 4 days) increased cell death was evident in the HIV-1 exposed DC cultures (**papers I-II**). Overall, PDCs were found to be more sensitive to long-term culture than MDCs regardless of virus exposure. HIV-1 did not induce cell death in MDCs and PDCs as an instant effect of in vitro infection. However, one could of course question how this corresponds to the in vivo situation. Another theory to the loss of DCs in blood during HIV-1 infection is that DCs become activated by HIV-1, mature and migrate and are redistributed in the body. Our data may indirectly support this theory, as we detected an increased expression of co-stimulatory molecules CD86 (on MDCs and PDCs) as well as of CD40 and CD83 (on PDCs) after HIV-1 exposure (**paper II**). However, we did not observe upregulation of the homing receptor for migration to lymph nodes, CCR7, on MDCs and PDCs after HIV-1 exposure alone, as reported by others (306).

Cytokine profile

Signaling via CD40 induces DCs to upregulate of co-stimulatory molecules and secrete pro-inflammatory cytokines like IL-12 (307-309). A hypothesis in the HIV-1 field has been that DCs from HIV-1 infected individuals are defective in their ability to produce IL-12 and thereby fail to provide the help required for cytotoxic T cell responses. To address this, we studied the cytokine profile of HIV-1_{BaL} exposed MDDCs in response to CD40L stimulation. Analyses of the cell culture supernatants did not reveal any differences in the amounts of TNFα and IL-12 p70 secreted from HIV-1_{BaL} exposed or unexposed MDDCs stimulated with CD40L (**paper I**). However, intracellular cytokine stainings revealed that while TNFα was produced by both p24- and p24+ DCs after stimulation with CD40L, IL-12 p70 was exclusively produced by the p24- DCs. This emphasizes the difference between studying effects of CD40L on cytokine production in the total culture (where no differences were found between HIV-1 exposed and unexposed DCs) and the cytokine profile in individual cells. This is especially important in HIV-1 infection where a relatively small fraction of the DCs is infected. Thus, by applying intracellular staining to study the expression of cytokines and HIV-1 p24 at the single cell level, we were able to determine that while HIV-1_{BaL} infection did not alter TNFα production in response to CD40L stimulation it did interfere with IL-12 p70 production (**paper I**). Our data therefore indicate that HIV-

1 infected DCs are impaired in their ability to produce IL-12. Downmodulation of IL-12 may be a common strategy by viruses to evade the host immune system. Suppression of IL-12 has been documented in other viral infections like herpes virus, CMV and measles infection (310-314). It has been shown that PBMCs isolated from AIDS patients have a severe defect in their IL-12 production (216, 217). This impairment could be overcome by addition of soluble CD40L to monocyte cultures from HIV-1 infected patients, indicating that this defect may be due to insufficient signalling from bystander CD40L+ T cells and not due to impairment of the IL-12 producing cells themselves (218). However, our data suggest that reconstitution of CD40L in HIV-1 infected individuals may not fully restore the function of HIV-1 infected DCs in terms of IL-12 p70 production.

Next, we extended these findings by showing that HIV-1 infected primary MDCs and PDCs also produce TNFα upon TLR ligation with R-848 (**paper II**). R-848 is a low molecular weight imidazoquino-like compound that signals through TLR7 and 8. Human MDCs and PDCs both express TLR7 and have been shown to upregulate cell surface markers and secrete cytokines upon R-848 stimulation (43). However, we failed to detect intracellular IL-12 p70 in R-848 stimulated MDCs and PDCs. Still, by ELISA we found that R-848 stimulation induced IL-12 p70 secretion from MDCs, irrespective of HIV-1 exposure (43) and data not shown). Primary MDCs produce significantly less IL-12 p70 than MDDCs (315), which could explain why we detected IL-12 p70 intracellularly in MDDCs but not in MDCs and PDCs. Therefore, while we found that HIV-1 infected MDDCs are impaired in the ability to produce IL-12 p70 we cannot conclude that such a defect is present also in primary HIV-1 infected DCs. Primary DCs seem to retain functional capabilities despite HIV-1 infection. In deed, in our studies using autologous DC:CD4+ T cell co-cultures we did not see an impaired ability of HIV-1 exposed/infected primary MDCs and PDCs to induce recall T cell responses (**paper III**). However, it is possible that the function of HIV-1 infected DCs, as well as their potential impairment in cytokine production, is more critical for induction of primary T cell responses than for memory responses. Although we established productive HIV-1 infection in primary MDCs and PDCs, only 1-7% of the DCs were HIV-1 infected as determined by intracellular p24 staining. Dysfunctions in HIV-1 infected DCs may be difficult to detect, since the majority of the DCs in the cultures were uninfected (p24-).

We found that HIV-1 exposure induced production and secretion of IFNα from PDCs (**paper II**). It is well established that PDCs produce IFNα in response to exposure to different viruses like herpes simplex virus (HSV), influenza virus, Sendai virus as well as HIV-1 (199, 306, 316-319). It appears that exposure rather than infection is required for induction of IFNα production, as shown by the production of IFNα by PDCs that had been cultured in the presence of AZT, which inhibits productive infection. IFNα has a documented anti-viral effect (320-323). However, somewhat at odds with the antiviral effect of IFNα, we saw a positive correlation between the frequencies of HIV-1 infected p24+ PDCs and the levels of IFNα detected in the cell culture supernatant after 72 hours of exposure to HIV-1_{BaL}. Perhaps higher concentration of viral antigen in the cell culture (as a consequence of higher infection rate) resulted in induction of more IFNα. Intracellular IFNα stainings of HIV-1 infected PDCs were not performed in our study but one could speculate that the PDCs that are HIV-1 infected do not produce IFNα and vice versa PDCs that produce IFNα would not become infected.

The function of MDDCs vs. primary DCs after HIV-1 infection

Although MDDCs resemble primary MDCs with respect to cell surface markers and their response to stimuli, they are phenotypically and functionally distinct DC populations in a number of ways. While both subsets express myeloid markers like CD11c, MDDCs are often characterized by their expression of CD1a, which is not expressed by MDCs. On the other hand MDCs express CD1d, which MDDCs lack (315). The two DC populations express overlapping but not identical TLRs; MDDCs express high levels of TLR4 but no TLR7, while MDCs express TLR7 and lower levels of TLR4 (41, 42, 55, 59, 272, 324). MDCs and MDDCs are different also with respect to their migratory capacity, ability to secrete cytokines like IL-6, IL-10 and IL-12 p70 in response to various stimuli as well as their capacity to induce cytokine production in T cells (315, 325, 326). We found several marked differences between the MDDCs and primary MDCs, although we did not compare the DC subsets side by side. While MDDCs produce high levels of IL-12 p70 in response to CD40L (**paper I**), the levels of IL-12 p70 produced by MDCs in response to R-848 stimulation was much lower and not detectable by intracellular cytokine staining and flow cytometry (**paper II**). However, CD40L and R-848 stimulation are two different stimuli, reported

to induce different levels of IL-12 p70 in MDDCs (327). Comparing MDCs and MDDCs from the same donor, MDCs were found to produce less IL-12 p70 in response to different stimuli than MDDCs (315). Still, our intracellular analyses of cytokine production in MDDCs after HIV-1 infection and the observed inability of HIV-1 infected p24+ MDDCs to produce IL-12 p70 illustrates a possible mechanism to explain the inadequate T cell responses observed in vivo.

HIV-1 exposure of MDCs, on the other hand, resulted in upregulation of the co-stimulatory molecule CD86 but not CD40 and CD83 (**paper II**). In contrast, the bulk culture of MDDCs did not mature after HIV-1 exposure alone, but a slight upregulation of co-stimulatory molecules was only seen on the fraction of MDDCs that were HIV-1 infected (**paper I**). However, stimulation resulted in full maturation as defined by a marked increase in the expression of these cell surface markers; both in HIV-1 exposed an unexposed MDDCs and MDCs (**papers I**-**II**). MDCs appear to be more sensitive to HIV-1 exposure than MDDCs, at least in terms of upregulation of cell surface markers (219, 304, 305). Also, MDCs are more efficient than MDDCs in polarizing proliferating T cells to a Th1 type response (325), despite that they produce less IL-12 compared to MDDCs. In addition, different protocols to generate MDDCs most likely result in different DC-like cells, depending on the source of serum, concentration and source of recombinant cytokines, cell density and length of culture, which complicate matters even more. These differences between in vitro and primary in vivo generated DCs are important to consider when interpreting data and consequently when choosing what type of DCs to use for potential clinical applications.

Taken together, our findings suggest HIV-1 infection has multiple effects on the functional properties of DCs, which could help provide insight in the full complexity of the contribution of DCs to the pathogenesis of the disease.

The role of dendritic cells in spreading HIV-1 infection

The migratory nature of DCs and their susceptibility to bind to and become infected by HIV-1, along with their ability to interact with numerous T cells in the lymphoid tissue, identifies them as strong candidates for a central role in spreading HIV-1 in the host (328). After sexual transmission, HIV-1 crosses the mucosal epithelium and eventually ends up in the lymphoid tissue where a permanent infection is established

(329). Thus, DCs are proposed to play a central role in the early events of HIV-1 transmission by transporting the virus from the periphery to the lymphoid compartment. As DCs and T cells interact to generate immune responses, this interaction also serve as a ideal microenvironment for HIV-1 replication and transfer between cells (113). In experimental transmission of SIV across the vaginal epithelium of rhesus macaques, intraepithelial DCs become productively infected at a low level within 18 hours after inoculation of the virus (330). The DCs subsequently migrate to draining lymph nodes where they infect resident CD4+ T cells (330). Naïve T cells require specific activation signals from antigen-presenting DCs in order to initiate their expansion and differentiation into memory and effector T cells and DCs possess the ability to cluster with numerous T cells to facilitate clonal expansion during immune activation. It is also known that complexes of T cells and DCs dramatically increase HIV-1 production by the T cells, as activated T cells become highly susceptible to HIV-1 and enhance viral replication (113, 183, 184, 304, 331, 332). A study using highly enriched HIV-1 infected MDDCs showed that these cells were poor stimulators of allogeneic T cell proliferation and that HIV-1 infected DCs indeed may contribute to impaired T cell mediated immune responses (300). In our study, we analyzed recall CD4+ T cell responses against CMV and SEB (**paper III**). It would have been interesting to study transfer of HIV-1 to HIV-1 specific CD4+ T cells. However, this would have required the use of DCs and T cells isolated from HIV-1 infected individuals or the induction of primary HIV-1 specific immune responses. To mimic the HIV-1 transfer taking place in vivo between infected DCs and naïve T cells using a cell culture system, would require establishment of primary HIV-1 infection and expansion of HIV-1 specific T cells in vitro, which would be technically challenging.

As described earlier, we showed that both MDCs and PDCs are susceptible to productive infection with both R5-using HIV-1_{BaL} and X4-using HIV-1_{IIIB} and that there is a difference in susceptibility to HIV-1 infection between the two DC subsets (**paper II**). Next, we could show that both MDCs and PDCs were able to transfer the two HIV-1 isolates to autologous CD4+ T cells (**paper III**). To study the transfer of HIV-1 from infected DCs to T cells and not the following spread of virus between T cells and subsequent propagation of virus, we added Indinavir at the initiation of the co-culture. Indinavir is a protease inhibitor that binds to the active site of the viral protease and

thereby inhibits the proteolytic cleavage of the Gag and Gag-Pol precursors resulting in only non-infectious particles being produced. Consequently, addition of Indinavir to the co-cultures of HIV-1 infected DCs with autologous CD4+ T cells allowed transfer of the virus produced in the DCs prior to co-culture but no further spread of the virus. Addition of Indinavir substantially reduced the frequency of p24+ T cells, compared to the frequencies of p24+ T cells detected in the absence of Indinavir in the DC-T cell co-cultures. This shows that under normal conditions, i.e. in the absence of antiretroviral drugs, HIV-1 is mainly propagated by T cell to T cell spread after the initial transfer from DCs. With Indinavir present in the co-culture, significantly higher frequencies of p24+ T cells were found after co-culture with HIV-1_{BaL} exposed MDCs as compared to HIV-1_{IIIB} exposed MDCs. In contrast, HIV-1_{IIIB} exposed PDCs were again able to infect higher numbers of T cells as compared to HIV-1_{BaL} exposed PDCs. HIV-1_{IIIB} was transferred equally well by PDCs and MDCs. In contrast, HIV-1_{BaL} was more efficiently transferred to T cells by MDCs than PDCs. Taken together, these data indicate that there is a differential susceptibility and subsequent transfer of HIV-1_{BaL} and HIV-1_{IIIB} by the different DC subsets. This may play an important role in both the initial selection of HIV-1 strains after transmission as well as subsequent dissemination of virus in the infected host.

The role of DC-SIGN and productive infection of DCs in spreading HIV to T cells

HIV-1 interacts with DCs using several different pathways. In addition to the conventional CD4 and co-receptor mediated infection, HIV-1 can also bind to various CLRs on DCs without infecting the cells. The receptor studied in most detail in this respect is DC-SIGN, which is highly expressed on MDDCs. MDDCs can capture HIV-1 via gp120 binding to DC-SIGN expressed on the cell surface. The virus particle is then internalized into an early endosome and preserved intact and infectious. Upon contact with CD4+ T cells, DC-SIGN and virus is reexposed on the cell surface of the DCs and T cells may become infected (70, 333). DC-SIGN-bound virus markedly enhances infection of T cells in the presence of extremely small doses of virus; doses that normally would not infect T cells. Different subsets of DCs have been shown to express distinct arrays of CLRs that are capable of binding HIV-1 gp120 (49). Turville and co-workers investigated the expression of CLRs on LCs and DDCs, which are similar, but not identical, to the DC populations found in the squamous epithelia of the vaginal and anogenital tract. DC-SIGN was found only on CD14+ CD1a^{low} DDCs

and not on CD1a^{high} dermal DCs and LCs. LCs transfer HIV-1 to T cells in a CD4- and CCR5-dependent manner (334). However, LCs express langerin and DDCs express the mannose receptor, both known to bind gp120 (49). Therefore, if LCs, or the equivalent mucosal DCs, are the first cell to capture the virus, as they reside in the outer epithelium, other receptors than DC-SIGN must be involved. Once the virus reaches the submucosa, via LC transmission or through a damaged mucosal surface, DC-SIGN may play a role in spreading the virus. Interestingly, DDCs that migrate out of skin explants downregulated their expression of CLRs and gp120 binding becomes predominantly CD4 dependent (49). Although MDCs and PDCs express very low or undetectable levels of DC-SIGN (data not shown), other CLRs with a similar capacity to bind HIV-1 may be utilized. In order to examine the ability of MDCs and PDCs to transfer HIV-1 to CD4+ T cells without being productively infected themselves, we supplemented the cultures with AZT during the 72 hours of HIV-1 exposure to prevent productive infection of DCs (**paper III**). AZT was thereafter washed out together with residual virus before the DCs were co-cultured with autologous CD4+ T cells for 3.5 days. We found that transmission of virus to T cells by PDCs and MDCs required productive infection by the DCs. T cells co-culture with previously AZT treated DCs did not show any p24 expression. This was shown in T cells co-cultured with either PDCs or MDCs exposed to either HIV-1_{BaL} or HIV-1_{IIIB}. These data show that DC-mediated transfer of HIV-1 to T cells require productive HIV-1 infection of immature PDCs and MDCs and that no or undetectable levels of HIV-1 are transferred by other mechanisms in our co-culture system. The controversy of whether DCs must be productively infected to support transfer of virus to T cells may be dependent on the DC subset. It may also be a matter of time of viral exposure, as viral transfer from MDDCs to T cells is dependent on DC-SIGN early after HIV-1 exposure of the MDDCs (335). However, at later time points productive infection of MDDCs is required for transfer to T cells to occur (335). Here, we exposed the DCs to virus for 72 hours that resulted in productive infection of at least a fraction of the DCs, which could lessen the usage of DC-SIGN in this system. In addition, primary blood DCs may not the most relevant DC subset to study to determine the relevance of DC-SIGN mediated transfer of HIV-1 to T cells, since these DCs express very low or undetectable levels of DC-SIGN (**paper III**). Still, the relative contribution of these different pathways in vivo remains to be determined.

The nature of HIV-1 infected T cells after viral transfer from dendritic cells

DCs efficiently transfer HIV-1 to T cells. However, the exact nature of the T cells that become infected is still not known. It has been reported that HIV-1 preferentially infects CD4+ T cells specific for HIV-1 antigens as compared to T cells with other antigen specificities in infected individuals (172). Early studies showed that when DCs were pulsed with HIV-1 and presented an additional antigen (superantigen, alloantigen or tetanus toxoid); antigen-responding T cells appeared to be preferentially infected (170, 328, 336). Although HIV-1 preferentially infects antigen-specific T cells, other T cells are also targets for HIV-1 infection, possibly by transfer from DCs, and may function as viral reservoirs in infected individuals (184, 337, 338). We examined the CD4+ T cells that became infected by transfer from HIV-1 infected MDCs and PDCs during antigen presentation using a co-culture and flow cytometry model system that allowed detailed characterization of the DCs and T cells (**paper III**). As described above, we found that MDCs and PDCs efficiently transferred both an R5 and an X4 HIV-1 isolate to autologous CFSE-labeled CD4+ T cells. HIV-1 infected MDCs and PDCs presenting CMV antigens were also able to activate CMV-specific T cells to a similar extent as uninfected donor-matched DCs as measured by production of effector cytokines; IL-2, TNFα and IFNγ and proliferation. The responding antigen-specific T cells became HIV-1 infected more frequently as compared to non-responding T cells, i.e. T cells that did not divide and did not produce cytokines. In conclusion, both PDCs and MDCs preferentially transmitted HIV-1 to antigen-specific CD4+ T cells during DC-mediated activation of the T cells. Our results suggest that most DC-mediated HIV-1 infection of CD4+ T cells does not occur randomly. HIV-1 infected MDCs and PDCs presenting CMV antigens activated pre-existing autologous CMV-specific memory CD4+ T cells and preferentially transferred the virus to them. This pattern was also seen when using SEB and subsequent cross-linking of TCRs as an alternative to CMV antigens to activate T cells. This may help explain why HIV-1 specific T cells in infected individuals more frequently contain HIV-1 than T cells with other TCR-specificities (172).

Generation of HIV-1 specific immune responses after immunization with HIV-1 infected apoptotic cells

DCs present viral antigens to T cells after uptake of apoptotic cells derived from virus infected cells in vitro (274, 281-283, 339-342). However, it is unclear whether

apoptotic virus infected cells are capable of generating immune responses in vivo. To address the question whether HIV-1 infected apoptotic cells are capable of inducing HIV-1 specific immune responses in vivo, we used a pseudotype virus composed of the envelope of an amphotropic retrovirus murine leukemia virus (MuLV) and the HIV-1_{LAI} genome, to overcome the cellular tropism of HIV-1 and be able to use a mouse model to study HIV-1 immune responses (**paper IV**, (343)) (Figure 12). The pseudovirus was constructed by super-infection with HIV-1_{LAI} of a cell line persistently infected with MuLV (344, 345).

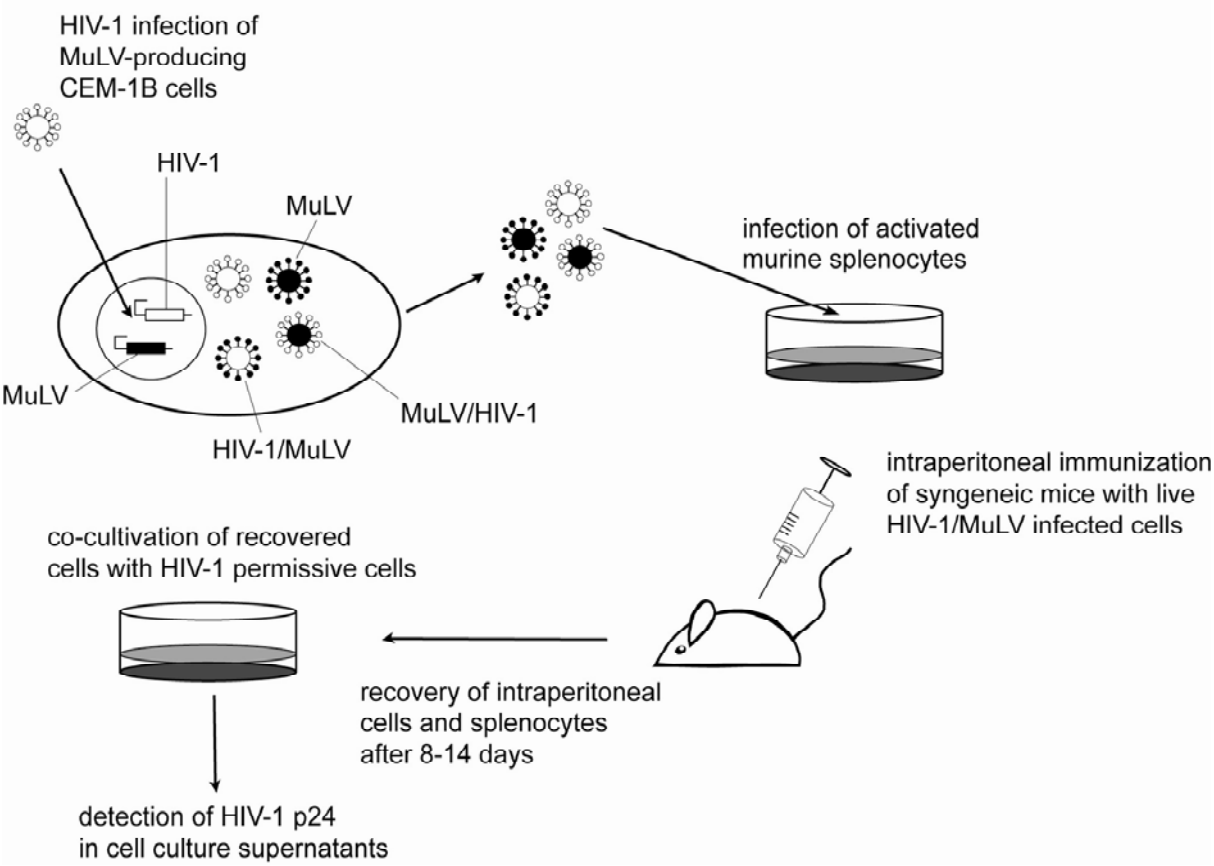

Figure 12. *A mouse model for HIV-1 infection. A human T cell line (CEM-1B) with an integrated MuLV, was super-infected with HIV-1_{Lai}. Upon infection and replication, the CEM cells produced a mixture of HIV-1, MuLV and HIV-1/MuLV chimeric virions. HIV-1/MuLV psudotype virus particles with the MuLV envelope infected activated murine splenocytes and the HIV-1 p24 content of infected cells was determined. The syngeneic HIV-1/MuLV infected splenocytes were injected in the peritoneal cavity of mice. After injection of live infected cells it was possible to detect infectious particles in the peritoneal cavity and spleen 8-14 days after inoculation, thereafter the cells are naturally cleared by the host. The level of HIV-1 in the collected tissues was determined by culture on HIV-1 permissive cells and measurement of secreted p24. Modified from* (346).

The supernatant collected contained a mixture of virus particles; MuLV, HIV-1, particles with the MuLV envelope containing the HIV-1 genome and vice versa and mosaic virus particles with HIV-1/MuLV envelope with either HIV-1 or MuLV genome. This supernatant was used to infect primary murine splenocytes from naïve mice. Only particles with the MuLV envelope were able to infect the splenocytes. Infected cells were assayed for the presence of HIV-1 by p24 ELISA (347). Batches of HIV/MuLV infected cells were frozen and used as source of apoptotic cells. Since the infection efficiency varied between different batches and in repeated experiments, the dose of 840±15 pg p24 per immunization was kept constant by varying the number of infected cells administrated (1-2x10^6 cells). Apoptosis was induced in infected splenocytes by γ-irradiation (348).

C57BL/6 mice were immunized intra-peritoneally (i.p.) according to the schedule in Figure 13, with apoptotic HIV-1/MuLV-infected or noninfected syngeneic splenocytes and subsequently challenged i.p. with live HIV-1/MuLV-infected splenocytes. We were not able to isolate replicating virus from the mice immunized with apoptotic HIV-1/MuLV infected cells, i.e. there was no release of infectious HIV-1 in the peritoneal cavity from the infected apoptotic cells.

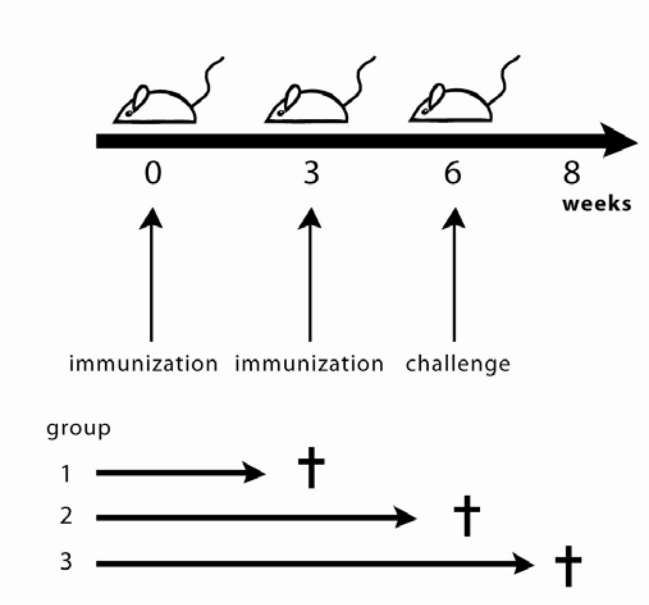

Figure 13. *Immunization(s) with apoptotic HIV-1/MuLV infected or noninfected cells and challenge with live HIV-1/MuLV infected cells.*

The i.p. immunization route was chosen to target antigens to the spleen. The spleen is a central immunological organ (349) and i.p. immunization potentially allows the study of antigen response in both B and T cells. The i.p. route can be questioned should this vaccination strategy be considered in human trials, but it is possible to

administrate vaccines i.p. also in humans (350). Still, additional studies in mice or non-human primates on alternative administration routes like intra-venously, intra-muscularly or intra-dermally, would be beneficial to improve this vaccine strategy.

We could show that immunizing mice with apoptotic HIV-1/MuLV infected cells induced HIV-1-specific immunity. Immunization with apoptotic HIV-1/MuLV-infected syngeneic splenocytes resulted in strong Nef-specific CD8+ T cell proliferation and p24 induced CD4+ and CD8+ T cell proliferation as well as IFNγ production. In addition, systemic HIV-1 specific IgG and IgA as well as mucosa-associated IgA responses were generated. Moreover, mice vaccinated with apoptotic HIV-1/MuLV cells cleared the challenge using live HIV-1/MuLV-infected cells, as no live HIV-1/MuLV-infected cells could be isolated from the peritoneal cavity of the mice 10 days after challenge. On the other hand, it was possible to isolate live HIV-1/MuLV-infected cells that could infect activated human T cells, from mice vaccinated with apoptotic noninfected (8/10) or MuLV-infected (3/4) splenocytes 10 days after challenge with live HIV-1/MuLV infected cells. This shows that immunization with apoptotic HIV-1/MuLV infected cells shifted the kinetics for clearing the infection in the mice as compared to mice immunized with apoptotic noninfected or MuLV infected cells.

The fact that we were able to induce HIV-1 specific immune responses using apoptotic HIV-1/MuLV infected cells without the use of an adjuvant implies that the apoptotic cells per se provided the necessary signals for immune activation. We hypothesize, although this remains to be established, that phagocytes in the peritoneal cavity, like macrophages or DCs, take up the apoptotic cells. The antigen presenting cells then mature and migrate to a nearby lymphoid site and present the antigen to T cells and generate the immune responses detected in the immunized mice.

It has been very difficult to generate antibodies that can neutralize primary isolates of HIV-1 (243). The generation of a strong humoral response with neutralizing antibodies is perhaps the most challenging part of generating an effective HIV-1 vaccine. We are encouraged by the broad systemic and mucosal-associated humoral HIV-1 specific immune response and the presence of cross-clade reactive antibodies

against the gp41 clade B/LAI peptide containing the ELDKWASLWN epitope, which is a part of a highly conserved broad HIV-1 subtype neutralizing epitope (351), after immunization with apoptotic HIV-1/MuLV infected cells. However, the potential HIV-1 neutralizing capacity of these antibodies remains to be determined.

After challenge with live HIV-1/MuLV infected cells in naïve mice it is possible to recover HIV-1/MuLV infected cells that can infect activated human T cells (as measured by p24 ELISA), from the peritoneal cavity and the spleen 8-14 days post inoculation, thereafter the infection is naturally cleared by the host (344, 345, 352). To study the potential long-term protective capacity of vaccination with apoptotic HIV-1/MuLV infected cells, these questions need to be addressed in a truly pathogenic animal model since HIV-1/MuLV has a limited replication capacity in mice as compared to SIV or SHIV in macaques (353). However, our data show that i.p. immunization with apoptotic HIV-1 infected cells induced HIV-1 specific systemic immune responses, primed for mucosal immune responses, and accelerated the clearance of challenge with live HIV-1 infected cells in mice.

These findings may have implications for the development of therapeutic and prophylactic HIV-1 vaccines. A therapeutic vaccine using this concept would include isolation of the circulating quasispecies of HIV-1 in the patient and subsequent superinfection of autologous cells with the patient’s own virus, which would hopefully overcome the problem with the high variability of HIV-1. However, this strategy requires modern laboratory facilities and trained personnel, which put demands on infrastructure. A prophylactic vaccine candidate based on this concept would perhaps use a cell line transfected with multiple truncated HIV-1 genes of different clades, coding for the known immunodominant epitopes of the various genes to obtain a broad response. This strategy excludes the potential benefit of using the patient’s own viral repertoire. On the other hand, such transfected cells could be obtained in large quantities and would be easier to distribute on a more extensive scale.

Concluding remarks

DCs are important in the generation of T cell responses. Still, it is unknown if DCs are as central to the pathogenesis of HIV-1 infection as they are for generating effective adaptive HIV-1 immune responses. Numerous parameters are involved in HIV-1 pathogenesis and contribute to disease progression in the infected host. The frequency of circulating DCs is reduced in HIV-1 infection and the reduction of DCs correlates with disease progression (176, 189, 207-213). Some data suggest that DCs are redistributed in the body and accumulate in lymphoid compartments in HIV-1 infected individuals (174). In addition, DCs isolated from HIV-1 seropositive persons have demonstrated a number of functional defects, like reduced capacity to induce T cell proliferation and decreased cytokine production as compared to DCs isolated from healthy donors (175-179). Based on these findings, we considered it important to study DCs in the context of HIV-1.

Here, we present data demonstrating an impaired ability of HIV-1 infected DCs to produce IL-12, an important cytokine in the initiation of cellular immune responses (**paper I**). In addition, we found that primary MDCs and PDCs were differentially susceptible to two different isolates of HIV-1 (**paper II**) and that productively infected DCs transferred HIV-1 primarily to antigen-reactivated CD4+ T cells (**paper III**). Furthermore, we showed that it was possible to induce HIV-1 specific immune responses in mice after immunization with apoptotic syngeneic HIV-1/MuLV infected cells and that these responses appeared to accelerate the ability of the mice to clear a challenge with live HIV-1/MuLV infected cells as compared to control animals (**paper IV**). Therefore, our data contribute both to an increased understanding of how DCs are affected by HIV-1 infection and subsequently provide insight as to how development of new strategies to combat HIV-1/AIDS could be developed.

Still many questions remain unanswered as to what the key elements in HIV-1 pathogenesis are and how we should address them. One strategy to obtain more knowledge on this subject is to continue the studies on the interaction between DCs and T cells during HIV-1 infection. It is important to understand how to obtain a balance between optimal induction of HIV-1 specific immune responses and reduced

risk of viral transfer and replication. In a continuation of the studies presented here, it would be beneficial to develop a procedure to isolate and examine pure populations of viable HIV-1 infected DCs. Pure populations of HIV-1 infected DCs would allow for a detailed study of how any impairments of HIV-1 infected DCs affect the induction of T cell responses. However, it is ultimately important to address the relative contribution of HIV-1 infected DCs to HIV-1 pathogenesis in vivo. One way to do that could be to study the transfer of HIV-1 from infected DCs to autologous HIV-1 specific T cells using blood or cells isolated from lymphoid compartments of HIV-1 infected individuals. Another way could be to take advantage of the SIV macaque models available. The use of apoptotic HIV-1 infected cells as a potential vaccine needs to be evaluated in an animal model where it is possible to establish an infection, like the SIV or SHIV infection in macaques. It is necessary to address if it is possible to administrate apoptotic infected cells without infecting and/or induce viral replication in the vaccinee. In addition, the immune responses elicited after immunization with apoptotic HIV-1/MuLV infected cells require more detailed study. Such efforts are currently made in the laboratory and the outcome of these experiments will be very interesting to follow.

An increased understanding of how DCs function under pathological and normal conditions is crucial to our understanding of immune defects in various diseases and I hope to continue my research on this topic in the future.

Populärvetenskaplig sammanfattning

I detta avhandlingsarbete har vi försökt förstå hur HIV påverkar dendritiska celler (DC). Vi har även undersökt möjligheten att använda döda HIV-infekterade celler som ett HIV-vaccin.

HIV är ett virus som sätter kroppens immunförsvar ur balans och långsamt bryter ner det. Sjukdomen AIDS uppstår när den HIV-infekterade personens immunförsvar är så försvagat att kroppen inte kan bekämpa andra infektioner som t.ex. lunginflammation. Världshälsoorganisationen WHO uppskattar att ungefär 38 miljoner människor i världen är smittade med HIV och cirka 14000 personer smittades varje dag under 2003. Idag finns det inget botemedel mot HIV och de mediciner som finns är tyvärr bara tillgängliga för en liten del av alla infekterade människor. Att utveckla effektivare mediciner och/eller ett HIV-vaccin är viktigt för att försöka hejda framfarten av HIV/AIDS i världen.

Vi har undersökt hur DC påverkas av HIV-infektion. DC är en typ av vita blodkroppar som har till uppgift att ta upp olika ämnen i kroppen, t.ex. virus och bakterier och visa upp dem för resten av immunsystemet. På så sätt kan kroppens immunförsvar känna igen och bekämpa sjukdomsalstrande organismer. DC kan bli infekterade av HIV, men de kan även bära med sig viruset när de förflyttar sig i kroppen, utan att själva bli infekterade. På så vis kan HIV snabbt och effektivt spridas i kroppen och komma i kontakt med ett stort antal celler som går att infektera. Dessutom kan HIV påverka DCs förmåga att på ett korrekt sätt visa övriga immunförsvaret att kroppen är infekterad med HIV och på så sätt hindra eller påverka immunförsvarets möjlighet att bekämpa infektionen.

Vi fann att HIV-infekterade DC är defekta i sin produktion av cytokinen IL-12, vilket är en substans som behövs för att generera s.k. cytotoxiska T-celler, en slags immunceller som känner igen och dödar virusinfekterade celler i kroppen. Att DC inte gör IL-12 när de är HIV-infekterade skulle kunna bidra till att färre cytotoxiska T-celler genereras och att kroppen därför har svårt att eliminera virusinfekterade celler. Vi fann även att när HIV-infekterade DC interagerar med T-celler, vilket är nödvändigt

för att kroppen ska starta ett immunförsvar, så överfördes viruset från DC till T-cellerna som då blev HIV-infekterade. Vi såg att det framförallt var de antigen-specifika T-cellerna, alltså de T-celler som kände igen de främmande ämnen som DC visade upp, som blev infekterade. Man har sett i HIV-infekterade personer att de T-celler som känner igen HIV oftare är HIV-infekterade än T-celler som känner igen andra ämnen. Det är allvarligt eftersom det är de HIV-specifika T-cellerna, som ska hjälpa till att döda de HIV-infekterade cellerna i kroppen. Våra resultat skulle kunna vara en del i förklaringen till varför just de HIV-specifika T-cellerna blir infekterade.

Vi har även undersökt om apoptotiska HIV-infekterade celler skulle kunna användas som ett HIV-vaccin. Apoptos kallas också programmerad celldöd och det är genom apoptos som celler normalt dör i kroppen när deras livstid är slut. Vid apoptos sönderdelas cellen i små ”cellpaket” (apoptotiska kroppar) som innehåller både genetisk information och proteiner. Vi vaccinerade möss med apoptotiska HIV-infekterade kroppar, som innehöll både HIV-gener och HIV-proteiner. De vaccinerade mössen började göra antikroppar som känner igen HIV och även HIV-specifika T-celler. Mössen verkade starta ett immunsvar mot HIV. När vi sedan sprutade in levande HIV-infekterade celler så kunde mössen som vaccinerats eliminera de infekterade cellerna fortare än möss som inte vaccinerats. De här resultaten är mycket lovande, men behöver studeras mer i detalj. Vi hoppas i framtiden kunna undersöka om apoptotiska HIV-infekterade celler är ett lika effektivt vaccin i människa som i mus.

Vi hoppas att våra resultat ska kunna hjälpa oss att förstå varför kroppen inte kan bekämpa HIV-infektion på ett effektivt sätt. Genom en större förståelse för vad som inte fungerar vid HIV-infektion kanske vi kan förstå vad som behöver korrigeras och hur vi ska göra det.

Acknowledgements

The work presented in this thesis was performed at the Center for Infectious Medicine (CIM) at Karolinska Institutet and at the Vaccine Research Center (VRC) at the National Institutes of Health. A number of people have contributed to this thesis and I would like to express my sincere gratitude to all who directly or indirectly have contributed to this work, in particular:

My supervisor, *Anna-Lena Spetz*, for initiating me into this project, for scientific support and for making me appreciate the importance of patience and hard work in science.

My co-supervisor, *Karin Loré*, for your belief in me and this project – it has been instrumental. Thank you for great times in and outside the lab, both in Stockholm and in Washington!

My co-supervisor, *Jan Andersson*, for inspiration and generosity in sharing your vast knowledge in immunology.

The *co-authors* to the papers included in this thesis; I would not have made it without you!

Hans-Gustaf Ljunggren, for creating an exciting and excellent scientific setting at CIM. For fruitful discussions and continuous encouragement.

Past and present members of the Spetz´ group: *Ulrika Johansson*, *Lilian Walther-Jallow*, *Shirin Heidari*, *Homira Behbahani* and *Ulrika Rolén*, for sharing the ups and downs in science, always willing to give a helping hand and a smile!

Annelie Tjernlund, for never-ending and enthusiastic help to find the way ahead!

Mattias Svensson and *Johan Sandberg* for critical reading of this thesis, for constructive, fun and inspiring discussions anytime, anywhere. Thanks!

Past and present members of CIM. *Kalle*, *Rickard*, *Alf*, *Benedict*, *Adnane*, *Antonio*, *Yenan* and *Markus* for motivating lunch discussions and good times. *Ann*, *Lena*, *Annette*, *Elisabeth* and *Hernán* for excellent administrative and technical assistance. The origin of CIM; *Jessica*, *Gail*, *Jakob*, *Anna NT*, *Máire*, *Arina*, *Pontus* and *Calle*. And of course; *Evren*, *Lisen*, *Steve*, *Erika*, *Mayte*, *Anna C*, *Arnaud*, *Barbro*, *Sirac*, *Daria*, *Malin*, *Johan F*, *Veronica*, *Sanna*, *Hayrettin*, *Niclas*, *Xiang*, *Kari*, *Michael*, *Monica*, *Stella*, *Liselotte*, *Kyriakos*, *Henrik*, *Lei*,

Mikolaj, *Abela*, *Annika O*, *Emma P*, *Cattis*, *Samer*, *Valli*, *Jun*, *Jonas*, *Beatrice*, *Karolin*, *Claudia*, *Alexandra*, *Christian*, *Mark (David)*, and *Hong*! I will miss you when I leave!

Richard Koup, for generously inviting me to the VRC and giving me excellent scientific training.

I would also like to thank *Bob Seder*, *John Mascola*, *Danny Douek*, and *Mario Roederer* at the VRC for nice discussions and for sharing your expertise in immunology with me. Sincere thanks to everyone at the VRC, especially; *David A*, *Charla*, *Mike*, *Jason*, *Jay*, *Pat*, *Joe*, *David C*, *Javier*, *Brenna*, *Janaki*, *Mark L*, *Steve P*, *David P*, *Laura*, *Ruth*, *Jessica*, *Ulli*, *Richard W*, *Masashi* and *the Immunology Core Lab*. Thank you for making my visits at the VRC exiting and productive – I hope to see you all again!

Sara Smed Sörensen, for excellent graphic design and daily help and encouragement during the finalization of this thesis.

Johan S, for company and discussions while working with the virus. The personnel at the Div. of Clinical Virology for practical guidance in the safety laboratory.

My friends. In particular, *Hanna*, my partner in crime during our graduate studies. *Annika*, for listening, laughing and concluding. *Lisa* and *Johan*, for good old (and future) times! The numerous *Algesten clan* (please, feel included), for your warm hearts and genuine interest in other people!

My family, to whom I dedicate this thesis. My *mother* and *father*, my first and continuous source of strength, support and inspiration. My sisters *Ida* and *Sara*, who over and over again prove to be the best sisters in the world! My brother *Jonas* and his family for creating a safe haven where I always feel welcome. *Leopold*, the most recent member of the family.
Finally, *Robert*, for love and support every day. You help me focus on the solution, not the problem, and that makes all the difference. Thank you.

References

1. Langerhans, P. 1868. Uber die Nerven der menschlien Haut. *Virch. Arch. (Pathol. Anat.) 44:325*.
2. Birbeck, M. S., Breathnach, A.S., Everal, J.D. 1961. An electron microscope study of basal melanocytes and high-level clear cells (Langerhans' cells) in vitiligo. *J Invest Dermatol 37:51*.
3. Takahashi, S., and K. Hashimoto. 1985. Derivation of Langerhans cell granules from cytomembrane. *J Invest Dermatol 84:469*.
4. Hashimoto, K. 1971. Langerhans' cell granule. An endocytotic organelle. *Arch Dermatol 104:148*.
5. Valladeau, J., C. Dezutter-Dambuyant, and S. Saeland. 2003. Langerin/CD207 sheds light on formation of birbeck granules and their possible function in Langerhans cells. *Immunol Res 28:93*.
6. Hoefsmit, E. C., B. M. Balfour, E. W. Kamperdijk, and J. Cvetanov. 1979. Cells containing Birbeck granules in the lymph and the lymph node. *Adv Exp Med Biol 114:389*.
7. Hoefsmit, E. C., A. M. Duijvestijn, and E. W. Kamperdijk. 1982. Relation between langerhans cells, veiled cells, and interdigitating cells. *Immunobiology 161:255*.
8. Hoshino, T., A. Kukita, and S. Sato. 1970. Cells containing Birbeck granules (Langerhans cell granules) in the human thymus. *J Electron Microsc (Tokyo) 19:271*.
9. Rowden, G., M. G. Lewis, and A. K. Sullivan. 1977. Ia antigen expression on human epidermal Langerhans cells. *Nature 268:247*.
10. Stingl, G., S. I. Katz, L. Clement, I. Green, and E. M. Shevach. 1978. Immunologic functions of Ia-bearing epidermal Langerhans cells. *J Immunol 121:2005*.
11. Tamaki, K., G. Stingl, M. Gullino, D. H. Sachs, and S. I. Katz. 1979. Ia antigens in mouse skin are predominantly expressed on Langerhans cells. *J Immunol 123:784*.
12. Braathen, L. R., and E. Thorsby. 1980. Studies on human epidermal Langerhans cells. I. Allo-activating and antigen-presenting capacity. *Scand J Immunol 11:401*.
13. Braathen, L. R., and E. Thorsby. 1983. Human epidermal Langerhans cells are more potent than blood monocytes in inducing some antigen-specific T-cell responses. *Br J Dermatol 108:139*.
14. Knight, S. C., B. M. Balfour, J. O'Brien, L. Buttifant, T. Sumerska, and J. Clarke. 1982. Role of veiled cells in lymphocyte activation. *Eur J Immunol 12:1057*.
15. Streilein, J. W., L. W. Lonsberry, and P. R. Bergstresser. 1982. Depletion of epidermal langerhans cells and Ia immunogenicity from tape-stripped mouse skin. *J Exp Med 155:863*.
16. Steinman, R. M., and Z. A. Cohn. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med 137:1142*.
17. Steinman, R. M., D. S. Lustig, and Z. A. Cohn. 1974. Identification of a novel cell type in peripheral lymphoid organs of mice. 3. Functional properties in vivo. *J Exp Med 139:1431*.
18. Steinman, R. M., and Z. A. Cohn. 1974. Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J Exp Med 139:380*.
19. Steinman, R. M., J. C. Adams, and Z. A. Cohn. 1975. Identification of a novel cell type in peripheral lymphoid organs of mice. IV. Identification and distribution in mouse spleen. *J Exp Med 141:804*.
20. Katz, S. I., K. Tamaki, and D. H. Sachs. 1979. Epidermal Langerhans cells are derived from cells originating in bone marrow. *Nature 282:324*.
21. Katz, S. I., K. D. Cooper, M. Iijima, and T. Tsuchida. 1985. The role of Langerhans cells in antigen presentation. *J Invest Dermatol 85:96s*.
22. Barclay, A. N., and G. Mayrhofer. 1981. Bone marrow origin of Ia-positive cells in the medulla rat thymus. *J Exp Med 153:1666*.
23. Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R. M. Steinman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med 176:1693*.
24. Inaba, K., R. M. Steinman, M. W. Pack, H. Aya, M. Inaba, T. Sudo, S. Wolpe, and G. Schuler. 1992. Identification of proliferating dendritic cell precursors in mouse blood. *J Exp Med 175:1157*.
25. Caux, C., C. Dezutter-Dambuyant, D. Schmitt, and J. Banchereau. 1992. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. *Nature 360:258*.

26. Romani, N., S. Gruner, D. Brang, E. Kampgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P. O. Fritsch, R. M. Steinman, and G. Schuler. 1994. Proliferating dendritic cell progenitors in human blood. *J Exp Med 180:83*.
27. Reid, C. D., A. Stackpoole, A. Meager, and J. Tikerpae. 1992. Interactions of tumor necrosis factor with granulocyte-macrophage colony-stimulating factor and other cytokines in the regulation of dendritic cell growth in vitro from early bipotent CD34+ progenitors in human bone marrow. *J Immunol 149:2681*.
28. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med 179:1109*.
29. Young, J. W., P. Szabolcs, and M. A. Moore. 1995. Identification of dendritic cell colony-forming units among normal human CD34+ bone marrow progenitors that are expanded by c-kit-ligand and yield pure dendritic cell colonies in the presence of granulocyte/macrophage colony-stimulating factor and tumor necrosis factor alpha. *J Exp Med 182:1111*.
30. Grassi, F., C. Dezutter-Dambuyant, D. McIlroy, C. Jacquet, K. Yoneda, S. Imamura, L. Boumsell, D. Schmitt, B. Autran, P. Debre, and A. Hosmalin. 1998. Monocyte-derived dendritic cells have a phenotype comparable to that of dermal dendritic cells and display ultrastructural granules distinct from Birbeck granules. *J Leukoc Biol 64:484*.
31. Jaksits, S., E. Kriehuber, A. S. Charbonnier, K. Rappersberger, G. Stingl, and D. Maurer. 1999. CD34+ cell-derived CD14+ precursor cells develop into Langerhans cells in a TGF-beta 1-dependent manner. *J Immunol 163:4869*.
32. Strobl, H., E. Riedl, C. Scheinecker, C. Bello-Fernandez, W. F. Pickl, K. Rappersberger, O. Majdic, and W. Knapp. 1996. TGF-beta 1 promotes in vitro development of dendritic cells from CD34+ hemopoietic progenitors. *J Immunol 157:1499*.
33. Vremec, D., G. J. Lieschke, A. R. Dunn, L. Robb, D. Metcalf, and K. Shortman. 1997. The influence of granulocyte/macrophage colony-stimulating factor on dendritic cell levels in mouse lymphoid organs. *Eur J Immunol 27:40*.
34. Maraskovsky, E., K. Brasel, M. Teepe, E. R. Roux, S. D. Lyman, K. Shortman, and H. J. McKenna. 1996. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J Exp Med 184:1953*.
35. Fong, L., M. Mengozzi, N. W. Abbey, B. G. Herndier, and E. G. Engleman. 2002. Productive infection of plasmacytoid dendritic cells with human immunodeficiency virus type 1 is triggered by CD40 ligation. *J Virol 76:11033*.
36. Pulendran, B., J. Banchereau, S. Burkeholder, E. Kraus, E. Guinet, C. Chalouni, D. Caron, C. Maliszewski, J. Davoust, J. Fay, and K. Palucka. 2000. Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets in vivo. *J Immunol 165:566*.
37. Randolph, G. J., S. Beaulieu, S. Lebecque, R. M. Steinman, and W. A. Muller. 1998. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science 282:480*.
38. Loetze, M. T., Thomson, A.W. 2001. *Dendritic cells*. Academic Press, London.
39. Shortman, K., and Y. J. Liu. 2002. Mouse and human dendritic cell subtypes. *Nat Rev Immunol 2:151*.
40. Grouard, G., M. C. Rissoan, L. Filgueira, I. Durand, J. Banchereau, and Y. J. Liu. 1997. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med 185:1101*.
41. Jarrossay, D., G. Napolitani, M. Colonna, F. Sallusto, and A. Lanzavecchia. 2001. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur J Immunol 31:3388*.
42. Kadowaki, N., S. Ho, S. Antonenko, R. W. Malefyt, R. A. Kastelein, F. Bazan, and Y. J. Liu. 2001. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med 194:863*.
43. Lore, K., M. R. Betts, J. M. Brenchley, J. Kuruppu, S. Khojasteh, S. Perfetto, M. Roederer, R. A. Seder, and R. A. Koup. 2003. Toll-like receptor ligands modulate dendritic cells to augment cytomegalovirus- and HIV-1-specific T cell responses. *J Immunol 171:4320*.
44. Cella, M., D. Jarrossay, F. Facchetti, O. Alebardi, H. Nakajima, A. Lanzavecchia, and M. Colonna. 1999. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med 5:919*.
45. Siegal, F. P., N. Kadowaki, M. Shodell, P. A. Fitzgerald-Bocarsly, K. Shah, S. Ho, S. Antonenko, and Y. J. Liu. 1999. The nature of the principal type 1 interferon-producing cells in human blood. *Science 284:1835*.

46. Summers, K. L., B. D. Hock, J. L. McKenzie, and D. N. Hart. 2001. Phenotypic characterization of five dendritic cell subsets in human tonsils. *Am J Pathol 159:285*.
47. Res, P. C., F. Couwenberg, F. A. Vyth-Dreese, and H. Spits. 1999. Expression of pTalpha mRNA in a committed dendritic cell precursor in the human thymus. *Blood 94:2647*.
48. Romani, N., S. Holzmann, C. H. Tripp, F. Koch, and P. Stoitzner. 2003. Langerhans cells - dendritic cells of the epidermis. *Apmis 111:725*.
49. Turville, S. G., P. U. Cameron, A. Handley, G. Lin, S. Pohlmann, R. W. Doms, and A. L. Cunningham. 2002. Diversity of receptors binding HIV on dendritic cell subsets. *Nat Immunol 3:975*.
50. Sallusto, F. 2001. Origin and migratory properties of dendritic cells in the skin. *Curr Opin Allergy Clin Immunol 1:441*.
51. McLellan, A. D., A. Heiser, R. V. Sorg, D. B. Fearnley, and D. N. Hart. 1998. Dermal dendritic cells associated with T lymphocytes in normal human skin display an activated phenotype. *J Invest Dermatol 111:841*.
52. Takeda, K., T. Kaisho, and S. Akira. 2003. Toll-like receptors. *Annu Rev Immunol 21:335*.
53. Janeway, C. A., Jr., and R. Medzhitov. 1998. Introduction: the role of innate immunity in the adaptive immune response. *Semin Immunol 10:349*.
54. Muzio, M., D. Bosisio, N. Polentarutti, G. D'Amico, A. Stoppacciaro, R. Mancinelli, C. van't Veer, G. Penton-Rol, L. P. Ruco, P. Allavena, and A. Mantovani. 2000. Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. *J Immunol 164:5998*.
55. Krug, A., A. Towarowski, S. Britsch, S. Rothenfusser, V. Hornung, R. Bals, T. Giese, H. Engelmann, S. Endres, A. M. Krieg, and G. Hartmann. 2001. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur J Immunol 31:3026*.
56. Bauer, M., V. Redecke, J. W. Ellwart, B. Scherer, J. P. Kremer, H. Wagner, and G. B. Lipford. 2001. Bacterial CpG-DNA triggers activation and maturation of human CD11c-, CD123+ dendritic cells. *J Immunol 166:5000*.
57. Heil, F., H. Hemmi, H. Hochrein, F. Ampenberger, C. Kirschning, S. Akira, G. Lipford, H. Wagner, and S. Bauer. 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science 303:1526*.
58. Hemmi, H., T. Kaisho, O. Takeuchi, S. Sato, H. Sanjo, K. Hoshino, T. Horiuchi, H. Tomizawa, K. Takeda, and S. Akira. 2002. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat Immunol 3:196*.
59. Ito, T., R. Amakawa, T. Kaisho, H. Hemmi, K. Tajima, K. Uehira, Y. Ozaki, H. Tomizawa, S. Akira, and S. Fukuhara. 2002. Interferon-alpha and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. *J Exp Med 195:1507*.
60. Jurk, M., F. Heil, J. Vollmer, C. Schetter, A. M. Krieg, H. Wagner, G. Lipford, and S. Bauer. 2002. Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. *Nat Immunol 3:499*.
61. Diebold, S. S., T. Kaisho, H. Hemmi, S. Akira, and C. Reis e Sousa. 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science 303:1529*.
62. Drickamer, K. 1999. C-type lectin-like domains. *Curr Opin Struct Biol 9:585*.
63. Peiser, M., R. Wanner, and G. Kolde. 2004. Human epidermal Langerhans cells differ from monocyte-derived Langerhans cells in CD80 expression and in secretion of IL-12 after CD40 cross-linking. *J Leukoc Biol 76:616*.
64. Figdor, C. G., Y. van Kooyk, and G. J. Adema. 2002. C-type lectin receptors on dendritic cells and Langerhans cells. *Nat Rev Immunol 2:77*.
65. Engering, A., T. B. Geijtenbeek, S. J. van Vliet, M. Wijers, E. van Liempt, N. Demaurex, A. Lanzavecchia, J. Fransen, C. G. Figdor, V. Piguet, and Y. van Kooyk. 2002. The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J Immunol 168:2118*.
66. Geijtenbeek, T. B., R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, G. J. Adema, Y. van Kooyk, and C. G. Figdor. 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell 100:575*.
67. van Kooyk, Y., and T. B. Geijtenbeek. 2002. A novel adhesion pathway that regulates dendritic cell trafficking and T cell interactions. *Immunol Rev 186:47*.

68. Kato, M., T. K. Neil, D. B. Fearnley, A. D. McLellan, S. Vuckovic, and D. N. Hart. 2000. Expression of multilectin receptors and comparative FITC-dextran uptake by human dendritic cells. *Int Immunol 12:1511*.
69. Ebner, S., Z. Ehammer, S. Holzmann, P. Schwingshackl, M. Forstner, P. Stoitzner, G. M. Huemer, P. Fritsch, and N. Romani. 2004. Expression of C-type lectin receptors by subsets of dendritic cells in human skin. *Int Immunol 16:877*.
70. Geijtenbeek, T. B., D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor, and Y. van Kooyk. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell 100:587*.
71. Turville, S. G., J. Arthos, K. M. Donald, G. Lynch, H. Naif, G. Clark, D. Hart, and A. L. Cunningham. 2001. HIV gp120 receptors on human dendritic cells. *Blood 98:2482*.
72. Lee, B., G. Leslie, E. Soilleux, U. O'Doherty, S. Baik, E. Levroney, K. Flummerfelt, W. Swiggard, N. Coleman, M. Malim, and R. W. Doms. 2001. cis Expression of DC-SIGN allows for more efficient entry of human and simian immunodeficiency viruses via CD4 and a coreceptor. *J Virol 75:12028*.
73. Alvarez, C. P., F. Lasala, J. Carrillo, O. Muniz, A. L. Corbi, and R. Delgado. 2002. C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. *J Virol 76:6841*.
74. Simmons, G., J. D. Reeves, C. C. Grogan, L. H. Vandenberghe, F. Baribaud, J. C. Whitbeck, E. Burke, M. J. Buchmeier, E. J. Soilleux, J. L. Riley, R. W. Doms, P. Bates, and S. Pohlmann. 2003. DC-SIGN and DC-SIGNR bind ebola glycoproteins and enhance infection of macrophages and endothelial cells. *Virology 305:115*.
75. Halary, F., A. Amara, H. Lortat-Jacob, M. Messerle, T. Delaunay, C. Houles, F. Fieschi, F. Arenzana-Seisdedos, J. F. Moreau, and J. Dechanet-Merville. 2002. Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. *Immunity 17:653*.
76. Gardner, J. P., R. J. Durso, R. R. Arrigale, G. P. Donovan, P. J. Maddon, T. Dragic, and W. C. Olson. 2003. L-SIGN (CD 209L) is a liver-specific capture receptor for hepatitis C virus. *Proc Natl Acad Sci U S A 100:4498*.
77. Lozach, P. Y., H. Lortat-Jacob, A. de Lacroix de Lavalette, I. Staropoli, S. Foung, A. Amara, C. Houles, F. Fieschi, O. Schwartz, J. L. Virelizier, F. Arenzana-Seisdedos, and R. Altmeyer. 2003. DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. *J Biol Chem 278:20358*.
78. Pohlmann, S., J. Zhang, F. Baribaud, Z. Chen, G. J. Leslie, G. Lin, A. Granelli-Piperno, R. W. Doms, C. M. Rice, and J. A. McKeating. 2003. Hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGNR. *J Virol 77:4070*.
79. Appelmelk, B. J., I. van Die, S. J. van Vliet, C. M. Vandenbroucke-Grauls, T. B. Geijtenbeek, and Y. van Kooyk. 2003. Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. *J Immunol 170:1635*.
80. Maeda, N., J. Nigou, J. L. Herrmann, M. Jackson, A. Amara, P. H. Lagrange, G. Puzo, B. Gicquel, and O. Neyrolles. 2003. The cell surface receptor DC-SIGN discriminates between Mycobacterium species through selective recognition of the mannose caps on lipoarabinomannan. *J Biol Chem 278:5513*.
81. Cambi, A., K. Gijzen, J. M. de Vries, R. Torensma, B. Joosten, G. J. Adema, M. G. Netea, B. J. Kullberg, L. Romani, and C. G. Figdor. 2003. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells. *Eur J Immunol 33:532*.
82. Brown, G. D., J. Herre, D. L. Williams, J. A. Willment, A. S. Marshall, and S. Gordon. 2003. Dectin-1 mediates the biological effects of beta-glucans. *J Exp Med 197:1119*.
83. Gantner, B. N., R. M. Simmons, S. J. Canavera, S. Akira, and D. M. Underhill. 2003. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J Exp Med 197:1107*.
84. Geijtenbeek, T. B., S. J. Van Vliet, E. A. Koppel, M. Sanchez-Hernandez, C. M. Vandenbroucke-Grauls, B. Appelmelk, and Y. Van Kooyk. 2003. Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med 197:7*.
85. Geijtenbeek, T. B., S. J. van Vliet, A. Engering, B. A. t Hart, and Y. van Kooyk. 2004. Self- and nonself-recognition by C-type lectins on dendritic cells. *Annu Rev Immunol 22:33*.
86. Guermonprez, P., J. Valladeau, L. Zitvogel, C. Thery, and S. Amigorena. 2002. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol 20:621*.

87. Germain, R. N., and D. H. Margulies. 1993. The biochemistry and cell biology of antigen processing and presentation. *Annu Rev Immunol 11:403*.
88. Heemels, M. T., and H. Ploegh. 1995. Generation, translocation, and presentation of MHC class I-restricted peptides. *Annu Rev Biochem 64:463*.
89. Wolf, P. R., and H. L. Ploegh. 1995. How MHC class II molecules acquire peptide cargo: biosynthesis and trafficking through the endocytic pathway. *Annu Rev Cell Dev Biol 11:267*.
90. Bevan, M. J. 1976. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. *J Exp Med 143:1283*.
91. Heath, W. R., and F. R. Carbone. 2001. Cross-presentation, dendritic cells, tolerance and immunity. *Annu Rev Immunol 19:47*.
92. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245*.
93. Macatonia, S. E., A. J. Edwards, and S. C. Knight. 1986. Dendritic cells and the initiation of contact sensitivity to fluorescein isothiocyanate. *Immunology 59:509*.
94. Macatonia, S. E., S. C. Knight, A. J. Edwards, S. Griffiths, and P. Fryer. 1987. Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. Functional and morphological studies. *J Exp Med 166:1654*.
95. Sallusto, F., and A. Lanzavecchia. 1999. Mobilizing dendritic cells for tolerance, priming, and chronic inflammation. *J Exp Med 189:611*.
96. Sozzani, S., P. Allavena, A. Vecchi, and A. Mantovani. 1999. The role of chemokines in the regulation of dendritic cell trafficking. *J Leukoc Biol 66:1*.
97. West, M. A., R. P. Wallin, S. P. Matthews, H. G. Svensson, R. Zaru, H. G. Ljunggren, A. R. Prescott, and C. Watts. 2004. Enhanced dendritic cell antigen capture via toll-like receptor-induced actin remodeling. *Science 305:1153*.
98. Kalinski, P., C. M. Hilkens, E. A. Wierenga, and M. L. Kapsenberg. 1999. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol Today 20:561*.
99. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran, and K. Palucka. 2000. Immunobiology of dendritic cells. *Annu Rev Immunol 18:767*.
100. Roy, M., T. Waldschmidt, A. Aruffo, J. A. Ledbetter, and R. J. Noelle. 1993. The regulation of the expression of gp39, the CD40 ligand, on normal and cloned CD4+ T cells. *J Immunol 151:2497*.
101. Regnault, A., D. Lankar, V. Lacabanne, A. Rodriguez, C. Thery, M. Rescigno, T. Saito, S. Verbeek, C. Bonnerot, P. Ricciardi-Castagnoli, and S. Amigorena. 1999. Fcgamma receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. *J Exp Med 189:371*.
102. Jurgens, M., A. Wollenberg, D. Hanau, H. de la Salle, and T. Bieber. 1995. Activation of human epidermal Langerhans cells by engagement of the high affinity receptor for IgE, Fc epsilon RI. *J Immunol 155:5184*.
103. Geissmann, F., P. Launay, B. Pasquier, Y. Lepelletier, M. Leborgne, A. Lehuen, N. Brousse, and R. C. Monteiro. 2001. A subset of human dendritic cells expresses IgA Fc receptor (CD89), which mediates internalization and activation upon cross-linking by IgA complexes. *J Immunol 166:346*.
104. Cella, M., A. Engering, V. Pinet, J. Pieters, and A. Lanzavecchia. 1997. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature 388:782*.
105. Caux, C., C. Massacrier, B. Vanbervliet, B. Dubois, C. Van Kooten, I. Durand, and J. Banchereau. 1994. Activation of human dendritic cells through CD40 cross-linking. *J Exp Med 180:1263*.
106. DeBenedette, M. A., A. Shahinian, T. W. Mak, and T. H. Watts. 1997. Costimulation of CD28- T lymphocytes by 4-1BB ligand. *J Immunol 158:551*.
107. Gramaglia, I., D. Cooper, K. T. Miner, B. S. Kwon, and M. Croft. 2000. Co-stimulation of antigen-specific CD4 T cells by 4-1BB ligand. *Eur J Immunol 30:392*.
108. Ohshima, Y., Y. Tanaka, H. Tozawa, Y. Takahashi, C. Maliszewski, and G. Delespesse. 1997. Expression and function of OX40 ligand on human dendritic cells. *J Immunol 159:3838*.
109. Steinman, R. M., and M. D. Witmer. 1978. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc Natl Acad Sci U S A 75:5132*.
110. Nussenzweig, M. C., and R. M. Steinman. 1980. Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. *J Exp Med 151:1196*.
111. Green, J., and R. Jotte. 1985. Interactions between T helper cells and dendritic cells during the rat mixed lymphocyte reaction. *J Exp Med 162:1546*.

112. Steinman, R. M., B. Gutchinov, M. D. Witmer, and M. C. Nussenzweig. 1983. Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J Exp Med 157:613*.
113. Pope, M., M. G. Betjes, N. Romani, H. Hirmand, P. U. Cameron, L. Hoffman, S. Gezelter, G. Schuler, and R. M. Steinman. 1994. Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. *Cell 78:389*.
114. Mosmann, T. R., and R. L. Coffman. 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol 7:145*.
115. Maldonado-Lopez, R., T. De Smedt, P. Michel, J. Godfroid, B. Pajak, C. Heirman, K. Thielemans, O. Leo, J. Urbain, and M. Moser. 1999. CD8alpha+ and CD8alpha- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J Exp Med 189:587*.
116. Belardelli, F. 1995. Role of interferons and other cytokines in the regulation of the immune response. *Apmis 103:161*.
117. D'Andrea, A., M. Rengaraju, N. M. Valiante, J. Chehimi, M. Kubin, M. Aste, S. H. Chan, M. Kobayashi, D. Young, and E. Nickbarg. 1992. Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. *J Exp Med 176:1387*.
118. Macatonia, S. E., N. A. Hosken, M. Litton, P. Vieira, C. S. Hsieh, J. A. Culpepper, M. Wysocka, G. Trinchieri, K. M. Murphy, and A. O'Garra. 1995. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. *J Immunol 154:5071*.
119. Gately, M. K., L. M. Renzetti, J. Magram, A. S. Stern, L. Adorini, U. Gubler, and D. H. Presky. 1998. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol 16:495*.
120. Bennett, S. R., F. R. Carbone, F. Karamalis, R. A. Flavell, J. F. Miller, and W. R. Heath. 1998. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature 393:478*.
121. Ridge, J. P., F. Di Rosa, and P. Matzinger. 1998. A conditioned dendritic cell can be a temporal bridge between a CD4+ T- helper and a T-killer cell. *Nature 393:474*.
122. Schoenberger, S. P., R. E. Toes, E. I. van der Voort, R. Offringa, and C. J. Melief. 1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature 393:480*.
123. Fernandez, N. C., A. Lozier, C. Flament, P. Ricciardi-Castagnoli, D. Bellet, M. Suter, M. Perricaudet, T. Tursz, E. Maraskovsky, and L. Zitvogel. 1999. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. *Nat Med 5:405*.
124. Fujii, S., K. Shimizu, M. Kronenberg, and R. M. Steinman. 2002. Prolonged IFN-gamma-producing NKT response induced with alpha-galactosylceramide-loaded DCs. *Nat Immunol 3:867*.
125. Steinman, R. M., D. Hawiger, and M. C. Nussenzweig. 2003. Tolerogenic dendritic cells. *Annu Rev Immunol 21:685*.
126. CDC. 1981. Kaposis sarcoma and Pneumocystis Carinii pneumonia among homosexual men. *Morbid Mortal Weekly Report 30:305*.
127. Gottlieb, M. S., R. Schroff, H. M. Schanker, J. D. Weisman, P. T. Fan, R. A. Wolf, and A. Saxon. 1981. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med 305:1425*.
128. Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science 220:868*.
129. Gallo, R. C., P. S. Sarin, E. P. Gelmann, M. Robert-Guroff, E. Richardson, V. S. Kalyanaraman, D. Mann, G. D. Sidhu, R. E. Stahl, S. Zolla-Pazner, J. Leibowitch, and M. Popovic. 1983. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). *Science 220:865*.
130. Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, and et al. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science 224:500*.
131. Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo. 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science 224:497*.

132. Sarngadharan, M. G., M. Popovic, L. Bruch, J. Schupbach, and R. C. Gallo. 1984. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. *Science 224:506*.
133. Schupbach, J., M. Popovic, R. V. Gilden, M. A. Gonda, M. G. Sarngadharan, and R. C. Gallo. 1984. Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. *Science 224:503*.
134. Coffin, J., A. Haase, J. A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt, and et al. 1986. Human immunodeficiency viruses. *Science 232:697.*
135. Sipsas, N. V., S. A. Kalams, A. Trocha, S. He, W. A. Blattner, B. D. Walker, and R. P. Johnson. 1997. Identification of type-specific cytotoxic T lymphocyte responses to homologous viral proteins in laboratory workers accidentally infected with HIV-1. *J Clin Invest 99:752*.
136. Montagnier, L. 2002. HISTORICAL ESSAY: A History of HIV Discovery. *Science 298:1727*.
137. Gallo, R. C. 2002. HISTORICAL ESSAY: The Early Years of HIV/AIDS. *Science 298:1728*.
138. Gao, F., E. Bailes, D. L. Robertson, Y. Chen, C. M. Rodenburg, S. F. Michael, L. B. Cummins, L. O. Arthur, M. Peeters, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1999. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. *Nature 397:436*.
139. Hahn, B. H., G. M. Shaw, K. M. De Cock, and P. M. Sharp. 2000. AIDS as a zoonosis: scientific and public health implications. *Science 287:607*.
140. Rambaut, A., D. Posada, K. A. Crandall, and E. C. Holmes. 2004. The causes and consequences of HIV evolution. *Nat Rev Genet 5:52*.
141. Santiago, M. L., C. M. Rodenburg, S. Kamenya, F. Bibollet-Ruche, F. Gao, E. Bailes, S. Meleth, S. J. Soong, J. M. Kilby, Z. Moldoveanu, B. Fahey, M. N. Muller, A. Ayouba, E. Nerrienet, H. M. McClure, J. L. Heeney, A. E. Pusey, D. A. Collins, C. Boesch, R. W. Wrangham, J. Goodall, P. M. Sharp, G. M. Shaw, and B. H. Hahn. 2002. SIVcpz in wild chimpanzees. *Science 295:465*.
142. Weiss, R. A., and R. W. Wrangham. 1999. From Pan to pandemic. *Nature 397:385*.
143. Zhu, T., B. T. Korber, A. J. Nahmias, E. Hooper, P. M. Sharp, and D. D. Ho. 1998. An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. *Nature 391:594*.
144. Gao, F., L. Yue, A. T. White, P. G. Pappas, J. Barchue, A. P. Hanson, B. M. Greene, P. M. Sharp, G. M. Shaw, and B. H. Hahn. 1992. Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature 358:495*.
145. Chen, Z., P. Zhou, D. D. Ho, N. R. Landau, and P. A. Marx. 1997. Genetically divergent strains of simian immunodeficiency virus use CCR5 as a coreceptor for entry. *J Virol 71:2705*.
146. Sharp, P. M., E. Bailes, F. Gao, B. E. Beer, V. M. Hirsch, and B. H. Hahn. 2000. Origins and evolution of AIDS viruses: estimating the time-scale. *Biochem Soc Trans 28:275*.
147. Gould, P. 1993. *The slow plague. A Geography of the AIDS Pandemic.* Blackwell, Cmbridge, MA.
148. Chitnis, A., D. Rawls, and J. Moore. 2000. Origin of HIV type 1 in colonial French Equatorial Africa? *AIDS Res Hum Retroviruses 16:5*.
149. Levy, J. A. 1993. Pathogenesis of human immunodeficiency virus infection. *Microbiol Rev 57:183*.
150. Vaishnav, Y. N., and F. Wong-Staal. 1991. The biochemistry of AIDS. *Annu Rev Biochem 60:577*.
151. Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier. 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature 312:767*.
152. Dalgleish, A. G., P. C. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss. 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature 312:763*.
153. Deng, H., R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, and N. R. Landau. 1996. Identification of a major co-receptor for primary isolates of HIV-1. *Nature 381:661*.
154. Berger, E. A., R. W. Doms, E. M. Fenyo, B. T. Korber, D. R. Littman, J. P. Moore, Q. J. Sattentau, H. Schuitemaker, J. Sodroski, and R. A. Weiss. 1998. A new classification for HIV-1. *Nature 391:240.*
155. Dragic, T., V. Litwin, G. P. Allaway, S. R. Martin, Y. Huang, K. A. Nagashima, C. Cayanan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton. 1996. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature 381:667*.

156. Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science 272:872*.
157. Frankel, A. D., and J. A. Young. 1998. HIV-1: fifteen proteins and an RNA. *Annu Rev Biochem 67:1*.
158. van't Wout, A. B., N. A. Kootstra, G. A. Mulder-Kampinga, N. Albrecht-van Lent, H. J. Scherpbier, J. Veenstra, K. Boer, R. A. Coutinho, F. Miedema, and H. Schuitemaker. 1994. Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical transmission. *J Clin Invest 94:2060*.
159. Zhang, L. Q., P. MacKenzie, A. Cleland, E. C. Holmes, A. J. Brown, and P. Simmonds. 1993. Selection for specific sequences in the external envelope protein of human immunodeficiency virus type 1 upon primary infection. *J Virol 67:3345*.
160. Zhu, T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup, and D. D. Ho. 1993. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. *Science 261:1179*.
161. Connor, R. I., K. E. Sheridan, D. Ceradini, S. Choe, and N. R. Landau. 1997. Change in coreceptor use coreceptor use correlates with disease progression in HIV-1--infected individuals. *J Exp Med 185:621*.
162. Koup, R. A., J. T. Safrit, Y. Cao, C. A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D. D. Ho. 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol 68:4650*.
163. McMichael, A. J. 1996. HIV. The immune response. *Curr Opin Immunol 8:537*.
164. McMichael, A. J., and S. L. Rowland-Jones. 2001. Cellular immune responses to HIV. *Nature 410:980*.
165. Mellors, J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science 272:1167*.
166. Ogg, G. S., X. Jin, S. Bonhoeffer, P. R. Dunbar, M. A. Nowak, S. Monard, J. P. Segal, Y. Cao, S. L. Rowland-Jones, V. Cerundolo, A. Hurley, M. Markowitz, D. D. Ho, D. F. Nixon, and A. J. McMichael. 1998. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science 279:2103*.
167. Johnson, R. P., R. F. Siliciano, and M. J. McElrath. 1998. Cellular immune responses to HIV-1. *Aids 12:S113*.
168. 2004. UNAIDS/WHO global AIDS statistics. *AIDS Care 16:788*.
169. Hellerstein, M., M. B. Hanley, D. Cesar, S. Siler, C. Papageorgopoulos, E. Wieder, D. Schmidt, R. Hoh, R. Neese, D. Macallan, S. Deeks, and J. M. McCune. 1999. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nat Med 5:83*.
170. Weissman, D., T. D. Barker, and A. S. Fauci. 1996. The efficiency of acute infection of CD4+ T cells is markedly enhanced in the setting of antigen-specific immune activation. *J Exp Med 183:687*.
171. Clerici, M., N. I. Stocks, R. A. Zajac, R. N. Boswell, D. R. Lucey, C. S. Via, and G. M. Shearer. 1989. Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients. Independence of CD4+ cell numbers and clinical staging. *J Clin Invest 84:1892*.
172. Douek, D. C., J. M. Brenchley, M. R. Betts, D. R. Ambrozak, B. J. Hill, Y. Okamoto, J. P. Casazza, J. Kuruppu, K. Kunstman, S. Wolinsky, Z. Grossman, M. Dybul, A. Oxenius, D. A. Price, M. Connors, and R. A. Koup. 2002. HIV preferentially infects HIV-specific CD4+ T cells. *Nature 417:95*.
173. Douek, D. C., R. D. McFarland, P. H. Keiser, E. A. Gage, J. M. Massey, B. F. Haynes, M. A. Polis, A. T. Haase, M. B. Feinberg, J. L. Sullivan, B. D. Jamieson, J. A. Zack, L. J. Picker, and R. A. Koup. 1998. Changes in thymic function with age and during the treatment of HIV infection. *Nature 396:690*.
174. Lore, K., A. Sonnerborg, C. Brostrom, L. E. Goh, L. Perrin, H. McDade, H. J. Stellbrink, B. Gazzard, R. Weber, L. A. Napolitano, Y. van Kooyk, and J. Andersson. 2002. Accumulation of DC-SIGN+CD40+ dendritic cells with reduced CD80 and CD86 expression in lymphoid tissue during acute HIV-1 infection. *Aids 16:683*.
175. Macatonia, S. E., S. Patterson, and S. C. Knight. 1989. Suppression of immune responses by dendritic cells infected with HIV. *Immunology 67:285*.
176. Macatonia, S. E., R. Lau, S. Patterson, A. J. Pinching, and S. C. Knight. 1990. Dendritic cell infection, depletion and dysfunction in HIV-infected individuals. *Immunology 71:38*.

177. Macatonia, S. E., M. Gompels, A. J. Pinching, S. Patterson, and S. C. Knight. 1992. Antigen-presentation by macrophages but not by dendritic cells in human immunodeficiency virus (HIV) infection. *Immunology 75:576*.
178. Knight, S. C., S. E. Macatonia, and S. Patterson. 1993. Infection of dendritic cells with HIV1: virus load regulates stimulation and suppression of T-cell activity. *Res Virol 144:75*.
179. Roberts, M., M. Gompels, A. J. Pinching, and S. C. Knight. 1994. Dendritic cells from HIV-1 infected individuals show reduced capacity to stimulate autologous T-cell proliferation. *Immunol Lett 43:39*.
180. Rubbert, A., C. Combadiere, M. Ostrowski, J. Arthos, M. Dybul, E. Machado, M. A. Cohn, J. A. Hoxie, P. M. Murphy, A. S. Fauci, and D. Weissman. 1998. Dendritic cells express multiple chemokine receptors used as coreceptors for HIV entry. *J Immunol 160:3933*.
181. Granelli-Piperno, A., B. Moser, M. Pope, D. Chen, Y. Wei, F. Isdell, U. O'Doherty, W. Paxton, R. Koup, S. Mojsov, N. Bhardwaj, I. Clark-Lewis, M. Baggiolini, and R. M. Steinman. 1996. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors. *J Exp Med 184:2433*.
182. Ayehunie, S., E. A. Garcia-Zepeda, J. A. Hoxie, R. Horuk, T. S. Kupper, A. D. Luster, and R. M. Ruprecht. 1997. Human immunodeficiency virus-1 entry into purified blood dendritic cells through CC and CXC chemokine coreceptors. *Blood 90:1379*.
183. Stahl-Hennig, C., R. M. Steinman, K. Tenner-Racz, M. Pope, N. Stolte, K. Matz-Rensing, G. Grobschupff, B. Raschdorff, G. Hunsmann, and P. Racz. 1999. Rapid infection of oral mucosal-associated lymphoid tissue with simian immunodeficiency virus. *Science 285:1261*.
184. Zhang, Z., T. Schuler, M. Zupancic, S. Wietgrefe, K. A. Staskus, K. A. Reimann, T. A. Reinhart, M. Rogan, W. Cavert, C. J. Miller, R. S. Veazey, D. Notermans, S. Little, S. A. Danner, D. D. Richman, D. Havlir, J. Wong, H. L. Jordan, T. W. Schacker, P. Racz, K. Tenner-Racz, N. L. Letvin, S. Wolinsky, and A. T. Haase. 1999. Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. *Science 286:1353*.
185. Tschachler, E., V. Groh, M. Popovic, D. L. Mann, K. Konrad, B. Safai, L. Eron, F. diMarzo Veronese, K. Wolff, and G. Stingl. 1987. Epidermal Langerhans cells--a target for HTLV-III/LAV infection. *J Invest Dermatol 88:233*.
186. Niedecken, H., G. Lutz, R. Bauer, and H. W. Kreysel. 1987. Langerhans cell as primary target and vehicle for transmission of HIV. *Lancet 2:519*.
187. von Stemm, A. M., J. Ramsauer, K. Tenner-Racz, H. F. Schmidt, I. Gigli, and P. Racz. 1993. Langerhans cells and interdigitating cells in HIV-infection. *Adv Exp Med Biol 329:539*.
188. Kalter, D. C., J. J. Greenhouse, J. M. Orenstein, S. M. Schnittman, H. E. Gendelman, and M. S. Meltzer. 1991. Epidermal Langerhans cells are not principal reservoirs of virus in HIV disease. *J Immunol 146:3396*.
189. Donaghy, H., B. Gazzard, F. Gotch, and S. Patterson. 2003. Dysfunction and infection of freshly isolated blood myeloid and plasmacytoid dendritic cells in patients infected with HIV-1. *Blood 101:4505*.
190. Cameron, P. U., U. Forsum, H. Teppler, A. Granelli-Piperno, and R. M. Steinman. 1992. During HIV-1 infection most blood dendritic cells are not productively infected and can induce allogeneic CD4+ T cells clonal expansion. *Clin Exp Immunol 88:226*.
191. Otero, M., G. Nunnari, D. Leto, J. Sullivan, F. X. Wang, I. Frank, Y. Xu, C. Patel, G. Dornadula, J. Kulkosky, and R. J. Pomerantz. 2003. Peripheral blood Dendritic cells are not a major reservoir for HIV type 1 in infected individuals on virally suppressive HAART. *AIDS Res Hum Retroviruses 19:1097*.
192. McIlroy, D., B. Autran, R. Cheynier, S. Wain-Hobson, J. P. Clauvel, E. Oksenhendler, P. Debre, and A. Hosmalin. 1995. Infection frequency of dendritic cells and CD4+ T lymphocytes in spleens of human immunodeficiency virus-positive patients. *J Virol 69:4737*.
193. Cameron, P. U., M. G. Lowe, S. M. Crowe, U. O'Doherty, M. Pope, S. Gezelter, and R. M. Steinman. 1994. Susceptibility of dendritic cells to HIV-1 infection in vitro. *J Leukoc Biol 56:257*.
194. Blauvelt, A. 1997. The role of skin dendritic cells in the initiation of human immunodeficiency virus infection. *Am J Med 102:16*.
195. Charbonnier, A. S., B. Verrier, C. Jacquet, C. Massacrier, M. M. Fiers, F. Mallet, C. Dezutter-Dambuyant, and D. Schmitt. 1996. In vitro HIV1 infection of CD34+ progenitor-derived dendritic/Langerhans cells at different stages of their differentiation in the presence of GM-CSF/TNF alpha. *Res Virol 147:89*.
196. Canque, B., Y. Bakri, S. Camus, M. Yagello, A. Benjouad, and J. C. Gluckman. 1999. The susceptibility to X4 and R5 human immunodeficiency virus-1 strains of dendritic cells derived

in vitro from CD34(+) hematopoietic progenitor cells is primarily determined by their maturation stage. *Blood 93:3866*.

197. Granelli-Piperno, A., E. Delgado, V. Finkel, W. Paxton, and R. M. Steinman. 1998. Immature dendritic cells selectively replicate macrophagetropic (M-tropic) human immunodeficiency virus type 1, while mature cells efficiently transmit both M- and T-tropic virus to T cells. *J Virol 72:2733*.
198. Patterson, S., A. Rae, N. Hockey, J. Gilmour, and F. Gotch. 2001. Plasmacytoid dendritic cells are highly susceptible to human immunodeficiency virus type 1 infection and release infectious virus. *J Virol 75:6710*.
199. Yonezawa, A., R. Morita, A. Takaori-Kondo, N. Kadowaki, T. Kitawaki, T. Hori, and T. Uchiyama. 2003. Natural alpha interferon-producing cells respond to human immunodeficiency virus type 1 with alpha interferon production and maturation into dendritic cells. *J Virol 77:3777*.
200. Stebbing, J., B. Gazzard, and D. C. Douek. 2004. Where does HIV live? *N Engl J Med 350:1872*.
201. Orenstein, J. M., C. Fox, and S. M. Wahl. 1997. Macrophages as a source of HIV during opportunistic infections. *Science 276:1857*.
202. Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci. 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science 233:1089*.
203. Livingstone, W. J., M. Moore, D. Innes, J. E. Bell, and P. Simmonds. 1996. Frequent infection of peripheral blood CD8-positive T-lymphocytes with HIV-1. Edinburgh Heterosexual Transmission Study Group. *Lancet 348:649*.
204. Motsinger, A., D. W. Haas, A. K. Stanic, L. Van Kaer, S. Joyce, and D. Unutmaz. 2002. CD1d-restricted human natural killer T cells are highly susceptible to human immunodeficiency virus 1 infection. *J Exp Med 195:869*.
205. Sandberg, J. K., N. M. Fast, E. H. Palacios, G. Fennelly, J. Dobroszycki, P. Palumbo, A. Wiznia, R. M. Grant, N. Bhardwaj, M. G. Rosenberg, and D. F. Nixon. 2002. Selective loss of innate CD4(+) V alpha 24 natural killer T cells in human immunodeficiency virus infection. *J Virol 76:7528*.
206. Valentin, A., M. Rosati, D. J. Patenaude, A. Hatzakis, L. G. Kostrikis, M. Lazanas, K. M. Wyvill, R. Yarchoan, and G. N. Pavlakis. 2002. Persistent HIV-1 infection of natural killer cells in patients receiving highly active antiretroviral therapy. *Proc Natl Acad Sci U S A 99:7015*.
207. Barron, M. A., N. Blyveis, B. E. Palmer, S. MaWhinney, and C. C. Wilson. 2003. Influence of plasma viremia on defects in number and immunophenotype of blood dendritic cell subsets in human immunodeficiency virus 1-infected individuals. *J Infect Dis 187:26*.
208. Chehimi, J., D. E. Campbell, L. Azzoni, D. Bacheller, E. Papasavvas, G. Jerandi, K. Mounzer, J. Kostman, G. Trinchieri, and L. J. Montaner. 2002. Persistent decreases in blood plasmacytoid dendritic cell number and function despite effective highly active antiretroviral therapy and increased blood myeloid dendritic cells in HIV-infected individuals. *J Immunol 168:4796*.
209. Feldman, S., D. Stein, S. Amrute, T. Denny, Z. Garcia, P. Kloser, Y. Sun, N. Megjugorac, and P. Fitzgerald-Bocarsly. 2001. Decreased interferon-alpha production in HIV-infected patients correlates with numerical and functional deficiencies in circulating type 2 dendritic cell precursors. *Clin Immunol 101:201*.
210. Feldman, S. B., M. C. Milone, P. Kloser, and P. Fitzgerald-Bocarsly. 1995. Functional deficiencies in two distinct interferon alpha-producing cell populations in peripheral blood mononuclear cells from human immunodeficiency virus seropositive patients. *J Leukoc Biol 57:214*.
211. Grassi, F., A. Hosmalin, D. McIlroy, V. Calvez, P. Debre, and B. Autran. 1999. Depletion in blood CD11c-positive dendritic cells from HIV-infected patients. *Aids 13:759*.
212. Pacanowski, J., S. Kahi, M. Baillet, P. Lebon, C. Deveau, C. Goujard, L. Meyer, E. Oksenhendler, M. Sinet, and A. Hosmalin. 2001. Reduced blood CD123+ (lymphoid) and CD11c+ (myeloid) dendritic cell numbers in primary HIV-1 infection. *Blood 98:3016*.
213. Soumelis, V., I. Scott, F. Gheyas, D. Bouhour, G. Cozon, L. Cotte, L. Huang, J. A. Levy, and Y. J. Liu. 2001. Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. *Blood 98:906*.

214. Donaghy, H., A. Pozniak, B. Gazzard, N. Qazi, J. Gilmour, F. Gotch, and S. Patterson. 2001. Loss of blood CD11c(+) myeloid and CD11c(-) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load. *Blood 98:2574*.
215. Pacanowski, J., L. Develioglu, I. Kamga, M. Sinet, M. Desvarieux, P. M. Girard, and A. Hosmalin. 2004. Early Plasmacytoid Dendritic Cell Changes Predict Plasma HIV Load Rebound during Primary Infection. *J Infect Dis 190:1889*.
216. Marshall, J. D., J. Chehimi, G. Gri, J. R. Kostman, L. J. Montaner, and G. Trinchieri. 1999. The interleukin-12-mediated pathway of immune events is dysfunctional in human immunodeficiency virus-infected individuals. *Blood 94:1003*.
217. Chehimi, J., S. E. Starr, I. Frank, A. D'Andrea, X. Ma, R. R. MacGregor, J. Sennelier, and G. Trinchieri. 1994. Impaired interleukin 12 production in human immunodeficiency virus- infected patients. *J Exp Med 179:1361*.
218. Chougnet, C., E. Thomas, A. L. Landay, H. A. Kessler, S. Buchbinder, S. Scheer, and G. M. Shearer. 1998. CD40 ligand and IFN-gamma synergistically restore IL-12 production in HIV-infected patients. *Eur J Immunol 28:646*.
219. Chougnet, C., S. S. Cohen, T. Kawamura, A. L. Landay, H. A. Kessler, E. Thomas, A. Blauvelt, and G. M. Shearer. 1999. Normal immune function of monocyte-derived dendritic cells from HIV- infected individuals: implications for immunotherapy. *J Immunol 163:1666*.
220. Vanham, G., L. Penne, K. Fransen, L. Kestens, and M. De Brabander. 2000. HIV-associated dysfunction of in vitro IL-12 production depends on the nature of the stimulus and on the CD4 T-cell count of the patient. *Blood 95:2185*.
221. Kornbluth, R. S. 2000. The emerging role of CD40 ligand in HIV infection. *J Leukoc Biol 68:373*.
222. Chirmule, N., T. W. McCloskey, R. Hu, V. S. Kalyanaraman, and S. Pahwa. 1995. HIV gp120 inhibits T cell activation by interfering with expression of costimulatory molecules CD40 ligand and CD80 (B71). *J Immunol 155:917*.
223. Lieberman, J., P. Shankar, N. Manjunath, and J. Andersson. 2001. Dressed to kill? A review of why antiviral CD8 T lymphocytes fail to prevent progressive immunodeficiency in HIV-1 infection. *Blood 98:1667*.
224. Palella, F. J., Jr., K. M. Delaney, A. C. Moorman, M. O. Loveless, J. Fuhrer, G. A. Satten, D. J. Aschman, and S. D. Holmberg. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med 338:853*.
225. Autran, B., G. Carcelain, T. S. Li, C. Blanc, D. Mathez, R. Tubiana, C. Katlama, P. Debre, and J. Leibowitch. 1997. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science 277:112*.
226. Mocroft, A., S. Vella, T. L. Benfield, A. Chiesi, V. Miller, P. Gargalianos, A. d'Arminio Monforte, I. Yust, J. N. Bruun, A. N. Phillips, and J. D. Lundgren. 1998. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. *Lancet 352:1725*.
227. Pontesilli, O., S. Kerkhof-Garde, D. W. Notermans, N. A. Foudraine, M. T. Roos, M. R. Klein, S. A. Danner, J. M. Lange, and F. Miedema. 1999. Functional T cell reconstitution and human immunodeficiency virus-1-specific cell-mediated immunity during highly active antiretroviral therapy. *J Infect Dis 180:76*.
228. Rinaldo, C. R., Jr., J. M. Liebmann, X. L. Huang, Z. Fan, Q. Al-Shboul, D. K. McMahon, R. D. Day, S. A. Riddler, and J. W. Mellors. 1999. Prolonged suppression of human immunodeficiency virus type 1 (HIV-1) viremia in persons with advanced disease results in enhancement of CD4 T cell reactivity to microbial antigens but not to HIV-1 antigens. *J Infect Dis 179:329*.
229. Ho, D. D. 1995. Time to hit HIV, early and hard. *N Engl J Med 333:450*.
230. Oxenius, A., D. A. Price, H. F. Gunthard, S. J. Dawson, C. Fagard, L. Perrin, M. Fischer, R. Weber, M. Plana, F. Garcia, B. Hirschel, A. McLean, and R. E. Phillips. 2002. Stimulation of HIV-specific cellular immunity by structured treatment interruption fails to enhance viral control in chronic HIV infection. *Proc Natl Acad Sci U S A 99:13747*.
231. Youle, M., G. Janossy, W. Turnbull, R. Tilling, C. Loveday, A. Mocroft, M. Tyrer, S. Madge, D. Wilson, A. Dykhoff, M. Johnson, and A. N. Phillips. 2000. Changes in CD4 lymphocyte counts after interruption of therapy in patients with viral failure on protease inhibitor-containing regimens. Royal Free Centre for HIV Medicine. *Aids 14:1717*.
232. Allen, T. M., A. D. Kelleher, J. Zaunders, and B. D. Walker. 2002. STI and beyond: the prospects of boosting anti-HIV immune responses. *Trends Immunol 23:456*.

233. Rosenberg, E. S., M. Altfeld, S. H. Poon, M. N. Phillips, B. M. Wilkes, R. L. Eldridge, G. K. Robbins, R. T. D'Aquila, P. J. Goulder, and B. D. Walker. 2000. Immune control of HIV-1 after early treatment of acute infection. *Nature 407:523*.
234. Blankson, J. N., D. Persaud, and R. F. Siliciano. 2002. The challenge of viral reservoirs in HIV-1 infection. *Annu Rev Med 53:557*.
235. Carr, A., and D. A. Cooper. 2000. Adverse effects of antiretroviral therapy. *Lancet 356:1423*.
236. Carr, A., K. Samaras, A. Thorisdottir, G. R. Kaufmann, D. J. Chisholm, and D. A. Cooper. 1999. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor- associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet 353:2093*.
237. Jenner, E. 1798. *An inquiry into the Causes and Effects of Variolae Vaccinae, a Disease Discovered in some Western counties of England.* Sampson Low, London.
238. Pasteur, L. 1880. Communication faite à la Académie des sciences, séance du 26 avril 1880 à l'Académie de médicine, séance du 27 avril 1880. *Oeuvres de Pasteus VI:303*.
239. Pantaleo, G., and R. A. Koup. 2004. Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nat Med 10:806*.
240. Autran, B., G. Carcelain, B. Combadiere, and P. Debre. 2004. Therapeutic vaccines for chronic infections. *Science 305:205*.
241. McMichael, A., M. Mwau, and T. Hanke. 2002. Design and tests of an HIV vaccine. *Br Med Bull 62:87*.
242. Amara, R. R., and H. L. Robinson. 2002. A new generation of HIV vaccines. *Trends Mol Med 8:489*.
243. Parren, P. W., J. P. Moore, D. R. Burton, and Q. J. Sattentau. 1999. The neutralizing antibody response to HIV-1: viral evasion and escape from humoral immunity. *Aids 13:S137*.
244. Preston, B. D. 1997. Reverse transcriptase fidelity and HIV-1 variation. *Science 275:228*.
245. Rowland-Jones, S., J. Sutton, K. Ariyoshi, T. Dong, F. Gotch, S. McAdam, D. Whitby, S. Sabally, A. Gallimore, and T. Corrah. 1995. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. *Nat Med 1:59*.
246. Yang, O. O., and B. D. Walker. 1997. CD8+ cells in human immunodeficiency virus type I pathogenesis: cytolytic and noncytolytic inhibition of viral replication. *Adv Immunol 66:273*.
247. Levy, J. A., C. E. Mackewicz, and E. Barker. 1996. Controlling HIV pathogenesis: the role of the noncytotoxic anti-HIV response of CD8+ T cells. *Immunol Today 17:217*.
248. Rubbert, A., D. Weissman, C. Combadiere, K. A. Pettrone, J. A. Daucher, P. M. Murphy, and A. S. Fauci. 1997. Multifactorial nature of noncytolytic CD8+ T cell-mediated suppression of HIV replication: beta-chemokine-dependent and -independent effects. *AIDS Res Hum Retroviruses 13:63*.
249. Tomaras, G. D., S. F. Lacey, C. B. McDanal, G. Ferrari, K. J. Weinhold, and M. L. Greenberg. 2000. CD8+ T cell-mediated suppressive activity inhibits HIV-1 after virus entry with kinetics indicating effects on virus gene expression. *Proc Natl Acad Sci U S A 97:3503*.
250. Pal, R., A. Garzino-Demo, P. D. Markham, J. Burns, M. Brown, R. C. Gallo, and A. L. DeVico. 1997. Inhibition of HIV-1 infection by the beta-chemokine MDC. *Science 278:695*.
251. Garzino-Demo, A., A. L. DeVico, F. Cocchi, and R. C. Gallo. 1998. Beta-chemokines and protection from HIV type 1 disease. *AIDS Res Hum Retroviruses 14 Suppl 2:S177*.
252. Kalams, S. A., and B. D. Walker. 1998. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J Exp Med 188:2199*.
253. Lee, S. K., Z. Xu, J. Lieberman, and P. Shankar. 2002. The functional CD8 T cell response to HIV becomes type-specific in progressive disease. *J Clin Invest 110:1339*.
254. Betts, M. R., D. R. Ambrozak, D. C. Douek, S. Bonhoeffer, J. M. Brenchley, J. P. Casazza, R. A. Koup, and L. J. Picker. 2001. Analysis of total human immunodeficiency virus (HIV)-specific CD4(+) and CD8(+) T-cell responses: relationship to viral load in untreated HIV infection. *J Virol 75:11983*.
255. Day, C. L., A. K. Shea, M. A. Altfeld, D. P. Olson, S. P. Buchbinder, F. M. Hecht, E. S. Rosenberg, B. D. Walker, and S. A. Kalams. 2001. Relative dominance of epitope-specific cytotoxic T-lymphocyte responses in human immunodeficiency virus type 1-infected persons with shared HLA alleles. *J Virol 75:6279*.
256. Kuiken, C., R. Thakallapalli, A. Esklid, and A. de Ronde. 2000. Genetic analysis reveals epidemiologic patterns in the spread of human immunodeficiency virus. *Am J Epidemiol 152:814*.
257. Finzi, D., and R. F. Siliciano. 1998. Viral dynamics in HIV-1 infection. *Cell 93:665*.
258. Sarkar, J. K., A. C. Mitra, and M. K. Mukherjee. 1975. The minimum protective level of antibodies in smallpox. *Bull World Health Organ 52:307*.

259. Mack, T. M., J. Noble, Jr., and D. B. Thomas. 1972. A prospective study of serum antibody and protection against smallpox. *Am J Trop Med Hyg 21:214*.
260. Callan, M. F. 2003. The evolution of antigen-specific CD8+ T cell responses after natural primary infection of humans with Epstein-Barr virus. *Viral Immunol 16:3*.
261. Doherty, P. C., J. P. Christensen, G. T. Belz, P. G. Stevenson, and M. Y. Sangster. 2001. Dissecting the host response to a gamma-herpesvirus. *Philos Trans R Soc Lond B Biol Sci 356:581*.
262. Amara, R. R., F. Villinger, J. D. Altman, S. L. Lydy, S. P. O'Neil, S. I. Staprans, D. C. Montefiori, Y. Xu, J. G. Herndon, L. S. Wyatt, M. A. Candido, N. L. Kozyr, P. L. Earl, J. M. Smith, H. L. Ma, B. D. Grimm, M. L. Hulsey, J. Miller, H. M. McClure, J. M. McNicholl, B. Moss, and H. L. Robinson. 2001. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science 292:69*.
263. Barouch, D. H., S. Santra, J. E. Schmitz, M. J. Kuroda, T. M. Fu, W. Wagner, M. Bilska, A. Craiu, X. X. Zheng, G. R. Krivulka, K. Beaudry, M. A. Lifton, C. E. Nickerson, W. L. Trigona, K. Punt, D. C. Freed, L. Guan, S. Dubey, D. Casimiro, A. Simon, M. E. Davies, M. Chastain, T. B. Strom, R. S. Gelman, D. C. Montefiori, M. G. Lewis, E. A. Emini, J. W. Shiver, and N. L. Letvin. 2000. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. *Science 290:486*.
264. Belyakov, I. M., Z. Hel, B. Kelsall, V. A. Kuznetsov, J. D. Ahlers, J. Nacsa, D. I. Watkins, T. M. Allen, A. Sette, J. Altman, R. Woodward, P. D. Markham, J. D. Clements, G. Franchini, W. Strober, and J. A. Berzofsky. 2001. Mucosal AIDS vaccine reduces disease and viral load in gut reservoir and blood after mucosal infection of macaques. *Nat Med 7:1320*.
265. Rose, N. F., P. A. Marx, A. Luckay, D. F. Nixon, W. J. Moretto, S. M. Donahoe, D. Montefiori, A. Roberts, L. Buonocore, and J. K. Rose. 2001. An effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. *Cell 106:539*.
266. Shiver, J. W., T. M. Fu, L. Chen, D. R. Casimiro, M. E. Davies, R. K. Evans, Z. Q. Zhang, A. J. Simon, W. L. Trigona, S. A. Dubey, L. Huang, V. A. Harris, R. S. Long, X. Liang, L. Handt, W. A. Schleif, L. Zhu, D. C. Freed, N. V. Persaud, L. Guan, K. S. Punt, A. Tang, M. Chen, K. A. Wilson, K. B. Collins, G. J. Heidecker, V. R. Fernandez, H. C. Perry, J. G. Joyce, K. M. Grimm, J. C. Cook, P. M. Keller, D. S. Kresock, H. Mach, R. D. Troutman, L. A. Isopi, D. M. Williams, Z. Xu, K. E. Bohannon, D. B. Volkin, D. C. Montefiori, A. Miura, G. R. Krivulka, M. A. Lifton, M. J. Kuroda, J. E. Schmitz, N. L. Letvin, M. J. Caulfield, A. J. Bett, R. Youil, D. C. Kaslow, and E. A. Emini. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature 415:331*.
267. Pantaleo, G. 1997. How immune-based interventions can change HIV therapy. *Nat Med 3:483*.
268. Katlama, C., G. Carcelain, C. Duvivier, C. Chouquet, R. Tubiana, M. De Sa, L. Zagury, V. Calvez, B. Autran, and D. Costagliola. 2002. Interleukin-2 accelerates CD4 cell reconstitution in HIV-infected patients with severe immunosuppression despite highly active antiretroviral therapy: the ILSTIM study--ANRS 082. *Aids 16:2027*.
269. Emery, S., and H. C. Lane. 1996. Immune-based therapies in HIV infection: recent developments. *Aids 10:S159*.
270. Ahlers, J. D., I. M. Belyakov, M. Terabe, R. Koka, D. D. Donaldson, E. K. Thomas, and J. A. Berzofsky. 2002. A push-pull approach to maximize vaccine efficacy: abrogating suppression with an IL-13 inhibitor while augmenting help with granulocyte/macrophage colony-stimulating factor and CD40L. *Proc Natl Acad Sci U S A 99:13020*.
271. Landay, A., C. Chougnet, S. S. Frankel, F. Veronese, and G. M. Shearer. 2002. Antigen-presenting cells in HIV pathogenesis and therapy: summary of the October 17-18, 2002, Think Tank meeting. *Clin Immunol 103:243*.
272. Iwasaki, A., and R. Medzhitov. 2004. Toll-like receptor control of the adaptive immune responses. *Nat Immunol 5:987*.
273. Akira, S., K. Takeda, and T. Kaisho. 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol 2:675*.
274. Engelmayer, J., M. Larsson, A. Lee, M. Lee, W. I. Cox, R. M. Steinman, and N. Bhardwaj. 2001. Mature dendritic cells infected with canarypox virus elicit strong anti- human immunodeficiency virus CD8+ and CD4+ T-cell responses from chronically infected individuals. *J Virol 75:2142*.
275. Granelli-Piperno, A., L. Zhong, P. Haslett, J. Jacobson, and R. M. Steinman. 2000. Dendritic cells, infected with vesicular stomatitis virus-pseudotyped HIV-1, present viral antigens to CD4+ and CD8+ T cells from HIV-1- infected individuals. *J Immunol 165:6620*.

276. Gruber, A., J. Kan-Mitchell, K. L. Kuhen, T. Mukai, and F. Wong-Staal. 2000. Dendritic cells transduced by multiply deleted HIV-1 vectors exhibit normal phenotypes and functions and elicit an HIV-specific cytotoxic T- lymphocyte response in vitro. *Blood 96:1327*.
277. Dhodapkar, M. V., and N. Bhardwaj. 2000. Active immunization of humans with dendritic cells. *J Clin Immunol 20:167*.
278. Lu, W., X. Wu, Y. Lu, W. Guo, and J. M. Andrieu. 2003. Therapeutic dendritic-cell vaccine for simian AIDS. *Nat Med 9:27*.
279. Piatak, M., Jr., M. S. Saag, L. C. Yang, S. J. Clark, J. C. Kappes, K. C. Luk, B. H. Hahn, G. M. Shaw, and J. D. Lifson. 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science 259:1749*.
280. Rodriguez, A., A. Regnault, M. Kleijmeer, P. Ricciardi-Castagnoli, and S. Amigorena. 1999. Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. *Nat Cell Biol 1:362*.
281. Subklewe, M., C. Paludan, M. L. Tsang, K. Mahnke, R. M. Steinman, and C. Munz. 2001. Dendritic cells cross-present latency gene products from Epstein-Barr virus-transformed B cells and expand tumor-reactive CD8(+) killer T cells. *J Exp Med 193:405*.
282. Albert, M. L., B. Sauter, and N. Bhardwaj. 1998. Dendritic cells acquire antigen from apoptotic cells and induce class I- restricted CTLs. *Nature 392:86*.
283. Arrode, G., C. Boccaccio, J. Lule, S. Allart, N. Moinard, J. P. Abastado, A. Alam, and C. Davrinche. 2000. Incoming human cytomegalovirus pp65 (UL83) contained in apoptotic infected fibroblasts is cross-presented to CD8(+) T cells by dendritic cells. *J Virol 74:10018*.
284. Robertson, J. D., S. Orrenius, and B. Zhivotovsky. 2000. Review: nuclear events in apoptosis. *J Struct Biol 129:346*.
285. Sauter, B., M. L. Albert, L. Francisco, M. Larsson, S. Somersan, and N. Bhardwaj. 2000. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J Exp Med 191:423*.
286. Zhao, X. Q., X. L. Huang, P. Gupta, L. Borowski, Z. Fan, S. C. Watkins, E. K. Thomas, and C. R. Rinaldo, Jr. 2002. Induction of anti-human immunodeficiency virus type 1 (HIV-1) CD8(+) and CD4(+) T-cell reactivity by dendritic cells loaded with HIV-1 X4- infected apoptotic cells. *J Virol 76:3007*.
287. Matzinger, P. 1994. Tolerance, danger, and the extended family. *Annu Rev Immunol 12:991*.
288. Matzinger, P. 1998. An innate sense of danger. *Semin Immunol 10:399*.
289. Medzhitov, R., and C. Janeway, Jr. 2000. Innate immune recognition: mechanisms and pathways. *Immunol Rev 173:89*.
290. Krieg, A. M. 1996. Lymphocyte activation by CpG dinucleotide motifs in prokaryotic DNA. *Trends Microbiol 4:73*.
291. Gallucci, S., M. Lolkema, and P. Matzinger. 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat Med 5:1249*.
292. Spetz, A. L., B. K. Patterson, K. Lore, J. Andersson, and L. Holmgren. 1999. Functional gene transfer of HIV DNA by an HIV receptor-independent mechanism. *J Immunol 163:736*.
293. Holmgren, L., A. Szeles, E. Rajnavolgyi, J. Folkman, G. Klein, I. Ernberg, and K. I. Falk. 1999. Horizontal transfer of DNA by the uptake of apoptotic bodies. *Blood 93:3956*.
294. Bergsmedh, A., A. Szeles, M. Henriksson, A. Bratt, M. J. Folkman, A. L. Spetz, and L. Holmgren. 2001. Horizontal transfer of oncogenes by uptake of apoptotic bodies. *Proc Natl Acad Sci U S A 98:6407*.
295. Robinson, H. L., D. C. Montefiori, R. P. Johnson, K. H. Manson, M. L. Kalish, J. D. Lifson, T. A. Rizvi, S. Lu, S. L. Hu, G. P. Mazzara, D. L. Panicali, J. G. Herndon, R. Glickman, M. A. Candido, S. L. Lydy, M. S. Wyand, and H. M. McClure. 1999. Neutralizing antibody-independent containment of immunodeficiency virus challenges by DNA priming and recombinant pox virus booster immunizations. *Nat Med 5:526*.
296. Schneider, J., S. C. Gilbert, C. M. Hannan, P. Degano, E. Prieur, E. G. Sheu, M. Plebanski, and A. V. Hill. 1999. Induction of CD8+ T cells using heterologous prime-boost immunisation strategies. *Immunol Rev 170:29*.
297. Barouch, D. H., J. Kunstman, M. J. Kuroda, J. E. Schmitz, S. Santra, F. W. Peyerl, G. R. Krivulka, K. Beaudry, M. A. Lifton, D. A. Gorgone, D. C. Montefiori, M. G. Lewis, S. M. Wolinsky, and N. L. Letvin. 2002. Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. *Nature 415:335*.

298. Lore, K., A. Sonnerborg, A. L. Spetz, U. Andersson, and J. Andersson. 1998. Erratum to "Immunocytochemical detection of cytokines and chemokines in Langerhans cells and in vitro derived dendritic cells". *J Immunol Methods 218:173*.
299. Brenchley, J. M. a. D., D.C. in press. *Cytometry: New Developments*. Academic Press, San Diego.
300. Kawamura, T., H. Gatanaga, D. L. Borris, M. Connors, H. Mitsuya, and A. Blauvelt. 2003. Decreased stimulation of CD4+ T cell proliferation and IL-2 production by highly enriched populations of HIV-infected dendritic cells. *J Immunol 170:4260*.
301. Reece, J. C., A. J. Handley, E. J. Anstee, W. A. Morrison, S. M. Crowe, and P. U. Cameron. 1998. HIV-1 selection by epidermal dendritic cells during transmission across human skin. *J Exp Med 187:1623*.
302. Patterson, B. K., A. Landay, J. Andersson, C. Brown, H. Behbahani, D. Jiyamapa, Z. Burki, D. Stanislawski, M. A. Czerniewski, and P. Garcia. 1998. Repertoire of chemokine receptor expression in the female genital tract: implications for human immunodeficiency virus transmission. *Am J Pathol 153:481*.
303. Meng, G., X. Wei, X. Wu, M. T. Sellers, J. M. Decker, Z. Moldoveanu, J. M. Orenstein, M. F. Graham, J. C. Kappes, J. Mestecky, G. M. Shaw, and P. D. Smith. 2002. Primary intestinal epithelial cells selectively transfer R5 HIV-1 to CCR5+ cells. *Nat Med 8:150*.
304. Canque, B., M. Rosenzwajg, S. Camus, M. Yagello, M. L. Bonnet, M. Guigon, and J. C. Gluckman. 1996. The effect of in vitro human immunodeficiency virus infection on dendritic-cell differentiation and function. *Blood 88:4215*.
305. Ghanekar, S., L. Zheng, A. Logar, J. Navratil, L. Borowski, P. Gupta, and C. Rinaldo. 1996. Cytokine expression by human peripheral blood dendritic cells stimulated in vitro with HIV-1 and herpes simplex virus. *J Immunol 157:4028*.
306. Fonteneau, J. F., M. Larsson, A. S. Beignon, K. McKenna, I. Dasilva, A. Amara, Y. J. Liu, J. D. Lifson, D. R. Littman, and N. Bhardwaj. 2004. Human immunodeficiency virus type 1 activates plasmacytoid dendritic cells and concomitantly induces the bystander maturation of myeloid dendritic cells. *J Virol 78:5223*.
307. Ebner, S., G. Ratzinger, B. Krosbacher, M. Schmuth, A. Weiss, D. Reider, R. A. Kroczek, M. Herold, C. Heufler, P. Fritsch, and N. Romani. 2001. Production of IL-12 by human monocyte-derived dendritic cells is optimal when the stimulus is given at the onset of maturation, and is further enhanced by IL-4. *J Immunol 166:633*.
308. Cella, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med 184:747*.
309. Steinman, R. M., M. Pack, and K. Inaba. 1997. Dendritic cell development and maturation. *Adv Exp Med Biol 417:1*.
310. Smith, A., F. Santoro, G. Di Lullo, L. Dagna, A. Verani, and P. Lusso. 2003. Selective suppression of IL-12 production by human herpesvirus 6. *Blood 102:2877*.
311. Servet-Delprat, C., P. O. Vidalain, H. Bausinger, S. Manie, F. Le Deist, O. Azocar, D. Hanau, A. Fischer, and C. Rabourdin-Combe. 2000. Measles virus induces abnormal differentiation of CD40 ligand-activated human dendritic cells. *J Immunol 164:1753*.
312. Moutaftsi, M., A. M. Mehl, L. K. Borysiewicz, and Z. Tabi. 2002. Human cytomegalovirus inhibits maturation and impairs function of monocyte-derived dendritic cells. *Blood 99:2913*.
313. Karp, C. L., M. Wysocka, L. M. Wahl, J. M. Ahearn, P. J. Cuomo, B. Sherry, G. Trinchieri, and D. E. Griffin. 1996. Mechanism of suppression of cell-mediated immunity by measles virus. *Science 273:228*.
314. Atabani, S. F., A. A. Byrnes, A. Jaye, I. M. Kidd, A. F. Magnusen, H. Whittle, and C. L. Karp. 2001. Natural measles causes prolonged suppression of interleukin-12 production. *J Infect Dis 184:1*.
315. Jefford, M., M. Schnurr, T. Toy, K. A. Masterman, A. Shin, T. Beecroft, T. Y. Tai, K. Shortman, M. Shackleton, I. D. Davis, P. Parente, T. Luft, W. Chen, J. Cebon, and E. Maraskovsky. 2003. Functional comparison of DCs generated in vivo with Flt3 ligand or in vitro from blood monocytes: differential regulation of function by specific classes of physiologic stimuli. *Blood 102:1753*.
316. Izaguirre, A., B. J. Barnes, S. Amrute, W. S. Yeow, N. Megjugorac, J. Dai, D. Feng, E. Chung, P. M. Pitha, and P. Fitzgerald-Bocarsly. 2003. Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. *J Leukoc Biol 74:1125*.

317. Lund, J., A. Sato, S. Akira, R. Medzhitov, and A. Iwasaki. 2003. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med 198:513*.
318. Fonteneau, J. F., M. Gilliet, M. Larsson, I. Dasilva, C. Munz, Y. J. Liu, and N. Bhardwaj. 2003. Activation of influenza virus-specific CD4+ and CD8+ T cells: a new role for plasmacytoid dendritic cells in adaptive immunity. *Blood 101:3520*.
319. Ferbas, J. J., J. F. Toso, A. J. Logar, J. S. Navratil, and C. R. Rinaldo, Jr. 1994. CD4+ blood dendritic cells are potent producers of IFN-alpha in response to in vitro HIV-1 infection. *J Immunol 152:4649*.
320. Stark, G. R., I. M. Kerr, B. R. Williams, R. H. Silverman, and R. D. Schreiber. 1998. How cells respond to interferons. *Annu Rev Biochem 67:227*.
321. Le Page, C., P. Genin, M. G. Baines, and J. Hiscott. 2000. Interferon activation and innate immunity. *Rev Immunogenet 2:374*.
322. Isaacs, A., and J. Lindenmann. 1957. Virus interference. I. The interferon. *Proc R Soc Lond B Biol Sci 147:258*.
323. Goodbourn, S., L. Didcock, and R. E. Randall. 2000. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. *J Gen Virol 81:2341*.
324. Visintin, A., A. Mazzoni, J. H. Spitzer, D. H. Wyllie, S. K. Dower, and D. M. Segal. 2001. Regulation of Toll-like receptors in human monocytes and dendritic cells. *J Immunol 166:249*.
325. Osugi, Y., S. Vuckovic, and D. N. Hart. 2002. Myeloid blood CD11c(+) dendritic cells and monocyte-derived dendritic cells differ in their ability to stimulate T lymphocytes. *Blood 100:2858*.
326. Luft, T., E. Maraskovsky, M. Schnurr, K. Knebel, M. Kirsch, M. Gorner, R. Skoda, A. D. Ho, P. Nawroth, and A. Bierhaus. 2004. Tuning the volume of the immune response: strength and persistence of stimulation determine migration and cytokine secretion of dendritic cells. *Blood 104:1066*.
327. Schnurr, M., T. Toy, A. Shin, M. Wagner, J. Cebon, and E. Maraskovsky. 2004. Extracellular nucleotide signalling via P2 receptors inhibits IL-12 and enhances IL-23 expression in human dendritic cells: a novel role for the cAMP pathway. *Blood*.
328. Cameron, P. U., P. S. Freudenthal, J. M. Barker, S. Gezelter, K. Inaba, and R. M. Steinman. 1992. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. *Science 257:383*.
329. Pantaleo, G., C. Graziosi, and A. S. Fauci. 1993. New concepts in the immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med 328:327*.
330. Hu, J., M. B. Gardner, and C. J. Miller. 2000. Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. *J Virol 74:6087*.
331. Frankel, S. S., B. M. Wenig, A. P. Burke, P. Mannan, L. D. Thompson, S. L. Abbondanzo, A. M. Nelson, M. Pope, and R. M. Steinman. 1996. Replication of HIV-1 in dendritic cell-derived syncytia at the mucosal surface of the adenoid. *Science 272:115*.
332. Pope, M., S. Gezelter, N. Gallo, L. Hoffman, and R. M. Steinman. 1995. Low levels of HIV-1 infection in cutaneous dendritic cells promote extensive viral replication upon binding to memory CD4+ T cells. *J Exp Med 182:2045*.
333. Kwon, D. S., G. Gregorio, N. Bitton, W. A. Hendrickson, and D. R. Littman. 2002. DC-SIGN-mediated internalization of HIV is required for trans-enhancement of T cell infection. *Immunity 16:135*.
334. Kawamura, T., S. S. Cohen, D. L. Borris, E. A. Aquilino, S. Glushakova, L. B. Margolis, J. M. Orenstein, R. E. Offord, A. R. Neurath, and A. Blauvelt. 2000. Candidate microbicides block HIV-1 infection of human immature Langerhans cells within epithelial tissue explants. *J Exp Med 192:1491*.
335. Turville, S. G., J. J. Santos, I. Frank, P. U. Cameron, J. Wilkinson, M. Miranda-Saksena, J. Dable, H. Stossel, N. Romani, M. Piatak, Jr., J. D. Lifson, M. Pope, and A. L. Cunningham. 2004. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. *Blood 103:2170*.
336. Cameron, P. U., M. Pope, S. Gezelter, and R. M. Steinman. 1994. Infection and apoptotic cell death of CD4+ T cells during an immune response to HIV-1-pulsed dendritic cells. *AIDS Res Hum Retroviruses 10:61*.
337. Siliciano, J. D., J. Kajdas, D. Finzi, T. C. Quinn, K. Chadwick, J. B. Margolick, C. Kovacs, S. J. Gange, and R. F. Siliciano. 2003. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med 9:727*.

338. Pierson, T., J. McArthur, and R. F. Siliciano. 2000. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. *Annu Rev Immunol 18:665*.
339. Ignatius, R., M. Marovich, E. Mehlhop, L. Villamide, K. Mahnke, W. I. Cox, F. Isdell, S. S. Frankel, J. R. Mascola, R. M. Steinman, and M. Pope. 2000. Canarypox virus-induced maturation of dendritic cells is mediated by apoptotic cell death and tumor necrosis factor alpha secretion. *J Virol 74:11329*.
340. Larsson, M., J. F. Fonteneau, M. Lirvall, P. Haslett, J. D. Lifson, and N. Bhardwaj. 2002. Activation of HIV-1 specific CD4 and CD8 T cells by human dendritic cells: roles for cross-presentation and non-infectious HIV-1 virus. *Aids 16:1319*.
341. Maranon, C., J. F. Desoutter, G. Hoeffel, W. Cohen, D. Hanau, and A. Hosmalin. 2004. Dendritic cells cross-present HIV antigens from live as well as apoptotic infected CD4+ T lymphocytes. *Proc Natl Acad Sci U S A 101:6092*.
342. Sigal, L. J., S. Crotty, R. Andino, and K. L. Rock. 1999. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. *Nature 398:77*.
343. Hinkula, J., E. Rollman, P. Lundholm, R. Benthin, K. Okuda, and B. Wahren. 2004. Genetic immunization with multiple HIV-1 genes provides protection against HIV-1/MuLV pseudovirus challenge in vivo. *Cells Tissues Organs 177:169*.
344. Andang, M., J. Hinkula, G. Hotchkiss, S. Larsson, S. Britton, F. Wong-Staal, B. Wahren, and L. Ahrlund-Richter. 1999. Dose-response resistance to HIV-1/MuLV pseudotype virus ex vivo in a hairpin ribozyme transgenic mouse model. *Proc Natl Acad Sci U S A 96:12749*.
345. Spector, D. H., E. Wade, D. A. Wright, V. Koval, C. Clark, D. Jaquish, and S. A. Spector. 1990. Human immunodeficiency virus pseudotypes with expanded cellular and species tropism. *J Virol 64:2298*.
346. Ljungberg, K. 2003. *Doctoral thesis*. Karolinska Institutet, Stockholm.
347. Devito, C., M. Levi, K. Broliden, and J. Hinkula. 2000. Mapping of B-cell epitopes in rabbits immunised with various gag antigens for the production of HIV-1 gag capture ELISA reagents. *J Immunol Methods 238:69*.
348. Maity, A., W. G. McKenna, and R. J. Muschel. 1994. The molecular basis for cell cycle delays following ionizing radiation: a review. *Radiother Oncol 31:1*.
349. Janeway, C. A., P. Travers, M. Walport, and M. Schlomchik. 2001. Immunobiology, the immune system in health and disease. Garland Publishing, New York.
350. Selgas, R., M. Fernandez de Castro, C. Jimenez, C. Carcamo, T. Contreras, M. A. Bajo, F. Vara, and A. Corbi. 1996. Immunomodulation of peritoneal macrophages by granulocyte-macrophage colony-stimulating factor in humans. *Kidney Int 50:2070*.
351. Broliden, P. A., A. von Gegerfelt, P. Clapham, J. Rosen, E. M. Fenyo, B. Wahren, and K. Broliden. 1992. Identification of human neutralization-inducing regions of the human immunodeficiency virus type 1 envelope glycoproteins. *Proc Natl Acad Sci U S A 89:461*.
352. Isaguliants, M. G., N. N. Petrakova, B. Zuber, K. Pokrovskaya, R. Gizatullin, D. A. Kostyuk, A. Kjerrstrom, G. Winberg, S. N. Kochetkov, J. Hinkula, and B. Wahren. 2000. DNA-encoding enzymatically active HIV-1 reverse transcriptase, but not the inactive mutant, confers resistance to experimental HIV-1 challenge. *Intervirology 43:288*.
353. Karlsson, G. B., M. Halloran, J. Li, I. W. Park, R. Gomila, K. A. Reimann, M. K. Axthelm, S. A. Iliff, N. L. Letvin, and J. Sodroski. 1997. Characterization of molecularly cloned simian-human immunodeficiency viruses causing rapid CD4+ lymphocyte depletion in rhesus monkeys. *J Virol 71:4218*.